NON-PATHOGENIC STRAINS OF HIV-1

CROSS REFERENCE TO RELATED APPLICATION

INS A

~~This application is a continuation-in-part application of US Serial No. 388,353 filed on 14 February, 1995.~~

FIELD OF THE INVENTION

The present invention relates to non-pathogenic strains of HIV-1 and to components, parts, fragments and derivatives thereof and to genetic sequences derived therefrom and their use in the development of diagnostic and therapeutic compositions for the treatment and prophylaxis of AIDS and AIDS-related disorders. The present invention also relates to a method for attenuating pathogenic strains of HIV-1 by mutagenizing particular regions of the HIV-1 genome. A particularly useful aspect of the present invention is a method for determining the likelihood or otherwise of an individual who is seropositive for HIV-1 developing AIDS or AIDS-related symptoms. Another aspect of the present invention is directed to strains of HIV-1 capable of synthesizing a modified Nef protein or having a wild-type Nef protein modified after synthesis thereby rendering those strains of HIV-1 substantially non-pathogenic.

BACKGROUND OF THE INVENTION

Bibliographic details of the publications referred to in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Genomic nucleotide sequences of HIV-1 strains referred to herein are represented by their corresponding DNA sequence.

Exemplary viral isolates referred to herein as "C18" and "C98" were deposited at the PHLS Centre for Applied Microbiology and Research, European Collection of Animal Cell Cultures (ECACC), Division of Biologies, Porton Down, Salisbury, Wiltshire SP4 OJG. C18 was deposited on 17 October, 1994 under Provisional Accession Number V94101706 and C98 was deposited on 31 October, 1994 under Provisional Accession Number V941031169. Viral isolate "C54" was deposited at ECACC on 10 March, 1995 under Provisional Accession Number V95031022.

A summary of particular deletion mutants of HIV-1 of the present invention referred to herein is given in Figure 11.

Acquired Immune Deficiency Syndrome (AIDS) and AIDS related disorders are the clinical result of infection by Human Immunodeficiency Virus type I (HIV-1) (Barre-Sinoussi *et al*, 1983). Infection by HIV-1 is generally characterised by progressive immune system damage (Teeuwsen *et al*, 1990; Clerici *et al*, 1989) leading to opportunistic infections, malignancies or wasting syndrome that constitute clinically-defined AIDS (Busch *et al*, 1991; Klaslow *et al*, 1990).

The high mortality rate of individuals infected with HIV-1 together with the social and economic consequences of the continuing HIV-1 epidemic has created an urgent need for a safe and effective treatment and/or prophylaxis against the devastating effects of AIDS. However, despite over a decade of high level scientific research into the pathogenesis of HIV-1 and the clinical manifestations of the disease, together with a detailed molecular analysis of the virus, there has been little success in the development of an effective vaccine. To date, the most effective therapy is treatment with zidovudine (AZT) which delays the onset of full blown AIDS and alleviates to some extent the symptoms f HIV-1 infection. However, AZT is not an innocuous compound and AZT, metabolic products thereof or impurities therein can cause a number f side effects

which limit long term treatment with the drug. Furthermore, AZT resistant isolates have been reported during treatment. Clearly, therefore, a need exists to develop alternative strategies in preventing and treating HIV-1 infection.

The initial phases of HIV-1 infection are summarised by Levy (1993) as involving attachment, fusion and nucleocapsid entry. These phases have been the traditional foci in research into development of antiviral strategies. The molecular events at the virus genomic level have also been the subject of intense scientific research with an aim being the development of a live attenuated vaccine as a possible approach for the treatment or prophylaxis of HIV-1 infection.

There is a high variable rate of progression from initial HIV-1 infection to AIDS which reflects a rapidly changing pathogen and variable immune response of the host to infection (Sheppard *et al*, 1993). With regards to the latter, HIV-1 can be considered as a heterogenous group of viruses differing at the genetic level with concomitant variable pathogenicity. For example, HIV-1 strains can differ in their capacity to kill cells. Furthermore, it appears that HIV-1 strains evolve in a host after infection and that the evolution varies depending on the tissues infected by the virus. The major sites in the genome apparently responsible for biological and pathological variation are the highly variable envelope region (Cheng-Mayer *et al*, 1991; Shioda *et al*, 1992; Hwang, *et al* 1991; Sullivan *et al*, 1993; Groenink *et al*, 1993) and the viral regulatory regions such as *tat* (Leguern *et al*, 1993). The genetic complexity of the HIV-1 group of viruses together with their variable pathogenicity, are major difficulties in the development f live vaccines, genetic vaccines or component vaccines.

Notwithstanding the highly pathogenic nature of HIV-1, there are some reports of long term survival of subjects infected with the virus (Learmont *et al*, 1992; Levy, 1993; Sheppard *et al*, 1993; Lifson *et al* 1991). It is not always clear, however, whether a benign course following HIV-1 infection is due to host factors, viral factors or other unknown factors. There are reports that most infected people have at least laboratory evidence f progressive immune system damage in the form of CD4+ cell loss (Lang *et al*, 1989) and d fective immune responses (Clerici *et al*, 1989).

Although simian monkeys have been used as an *in vivo* model for HIV and Simian Immunodeficiency Virus (SIV) infection, a major handicap in AIDS research has been the absence of suitable *in vivo* models to study the pathogenesis of the disease and, in particular, to study the viruses involved in benign infection. The need for a suitable *in vivo* model is heightened by the fact that results obtained *in vitro* cannot necessarily be extrapolated to what occurs *in vivo*. This was clearly observed by Mosier *et al* (1993) where conflicting results were obtained in animals compared to cell cultures.

Despite the absence of suitable *in vivo* models, considerable scientific research has been directed to attenuating HIV-1 strains by mutagenesis of the virus genome. Deletions in the *nef* gene have been implicated in attenuated strains of SIV and their use in providing protective effects in monkeys (Daniel *et al*, 1992). However, there are conflicting reports on the possible negative influence the *nef* gene product has on the rate or extent of virus replication (Terwilliger *et al*, 1986; Luciw *et al*, 1987; Niedermann *et al*, 1989; Kim *et al*, 1989; Hammes *et al*, 1989). In fact, Kim *et al* (1989) found that *nef* did not affect HIV-1 replication or HIV-1 long terminal repeat (LTR)-driven CAT expression. Kestler III *et al* (1991) found that the *nef* gene is required for full pathogenic potential in SIV. However, such is the complexity of the HIV-1 group of viruses and the variability of immune responses between individuals let alone different species, that it is far from clear whether *nef* deleted strains of HIV-1 would behave similarly to *nef* deleted strains of SIV-I. There is a need, therefore, in order to investigate the possibility of *nef* deleted HIV-1 strain as a vaccine candidate, to identify individuals infected with such modified viruses.

Learmont *et al* (1992) reported that a cohort of five persons infected with blood products from a single HIV-1 infected donor have remained asymptomatic from up to about 10-14 years after infection. Subsequently, a sixth person has been identified as being part of the cohort. Both the donor and recipients were HIV-1 seropositive but with no indications of clinical symptoms of HIV-1 related disease and CD4+ cell number and β_2-microglobulin levels have remained in the normal range. The identification of this cohort f benignly infected individuals provides a unique *in vivo* model in which the pathogenesis of HIV-1 infection can be studied at the clinical and molecular biological

levels.

However, it has not always been possible using conventional isolation procedures to routinely and reproducibly isolate viral strains from the above mentioned donor or recipients which has frustrated efforts to investigate the cause of the asymptomatic individuals. In accordance with the present invention, methods have now been established to isolate viruses from the above individuals. It has been determined, in accordance with the present invention, that individuals of the cohort are infected by non-pathogenic strains of HIV-1. Furthermore, the non-pathogenic strains of HIV-1 carry one or more nucleotide mutations. The non-pathogenic strains of the present invention enable the generation of a range of therapeutic, diagnostic and targeting agents against HIV-1 infection. The present invention also enables the attenuation of previously pathogenic strains of HIV-1. Additionally, an investigation of the immunological profiles of cohort individuals has revealed that a non-pathogenic strain of HIV-1 is indicated by a particular deletion in the coding region of a protein resulting in an altered immunological profile for the expressed protein. An example of an altered immunological profile results from a deletion of certain amino acids in the Nef protein.

SUMMARY OF THE INVENTION

One aspect of the invention is directed to an isolated HIV-1 strain or a component, part, fragment or derivative thereof which is substantially non-pathogenic.

Another aspect of the invention is directed to an isolated strain of HIV-1 or a biological source thereof, said HIV-1 having the following characteristics:

(i) is substantially non-pathogenic in human subjects; and

(ii) carries a modified *nef* gene which encodes a *nef* gene product substantially immunologically non-interactive with antibodies to amino acids 162 to 177 of Nef in wild-type HIV-1.

Yet another aspect f the invention is directed to an isolated non-pathogenic strain of HIV-1 comprising a genome which is substantially incapable f hybridizing under medium stringent conditi ns to a nucleic acid molecule comprising a sequence of

nucleotides which encodes all r part of amino acids 162 to 177 of wild-type HIV-1 Nef.

Still another aspect of the invention provides a non-pathogenic HIV-1 isolate which:

(i) induces an immune response in a human or primate subject;

(ii) does not substantially produce a proliferative response or cytokine production to a mitogen, alloantigen and/or recall antigen relative to a healthy, non-infected subject; and

(iii) is substantially incapable of inducing an antibody response to amino acids 162 to 177 of wild-type HIV-1 Nef protein.

Still yet another aspect of the invention contemplates a viral isolate which:

(i) is interactive to antibodies to a glycoprotein from HIV-1 selected from gp41-45, gp120 and gp160;

(ii) is substantially non-pathogenic in human subjects; and

(iii) carries a deletion mutation of at least ten nucleotides in a region corresponding to all or part of amino acids 162 to 177 encoded by the *nef* gene of a pathogenic strain of HIV-1.

Another aspect of the invention provides an isolated strain of HIV-1 which is reactive to antibodies to a glycoprotein of HIV-1, is capable of inducing an immune response to at least one of *gag*, *pol* and/or *env* and which is incapable of directing synthesis of a *nef* gene product or a full length *nef* gene product.

A further aspect of the invention contemplates a method for inhibiting or reducing productive infection of an individual by a pathogenic strain of HIV-1, said method comprising administering to a subject a non-pathogenic isolate of HIV-1 in an amount effective to infect target cells and to generate target cells carrying DNA derived from said non-pathogenic HIV-1.

In yet another aspect of the invention there is contemplated a method for vaccinating an individual against the development of AIDS or AIDS related diseases, said method comprising administering to said individual a non-pathogenic isolate of HIV-1 in an amount effective to infect target cells and to generate target cells carrying DNA derived from said non-pathogenic HIV-1.

In still yet another aspect of the invention there is provided a method for obtaining a preparation of non-pathogenic HIV-1 from a biological sample, said method comprising co-culturing PBMCs from said biological sample from an individual putatively infected with said non-pathogenic HIV-1 with HIV-1 seronegative donor PBMCs depleted for CD8+ cells, harvesting the PBMCs and supernatant fluid every from about 5 to about 10 days and adding fresh medium with CD8+ depleted PBMCs with said fresh medium and isolating said virus from the supernatant fluid.

Another aspect of the invention contemplates a method for obtaining a preparation of non-pathogenic HIV-1 from a biological sample, said method comprising co-culturing monocytes from said biological sample from an individual putatively infected with said non-pathogenic HIV-1 with HIV-1 seronegative donor PBMCs depleted for CD8+ cells, harvesting the monocytes and PBMCs and supernatant fluid every from about 5 to about 10 days and adding fresh medium with CD8+ depleted PBMCs with said fresh medium and isolating said virus from the supernatant fluid.

In yet a further aspect of the invention there is contemplated a method for identifying or screening for compounds capable of reducing or otherwise interfering with HIV-1 replication, said method comprising contacting a compound to be tested with a cell or cell extract containing or capable of containing a *nef* gene product fused to a reporter molecule capable of giving an identifiable signal and screening for a compound which inhibits said signal.

In still yet a further aspect of the invention there is provided a viral isolate which:

(i) is genetically or immunologically related to a pathogenic strain of HIV-1;

(ii) is substantially non-pathogenic in human subjects;

(iii) comprises a first nucleotide sequence constituting its genome which is capable of hybridising under medium stringency conditions to SEQ ID NO: 1 or a complementary form thereof; and

(iv) comprises a second nucleotide sequence within said first nucleotide sequence and which second nucleotide sequence directs expression f a mRNA molecule capable of inhibiting, reducing or otherwise down-regulating translation of a protein or polypeptide encoded by a pathogenic strain of HIV-1 or inhibit, reduce or otherwise down regulate operation of a non-protein encoding a region of a pathogenic strain of HIV-1.

Still yet another aspect of the invention is directed to a method for determining the pathogenicity of an HIV-1 strain after said HIV-1 strain infects cells of an individual, said method comprising determining the presence of a deletion mutation in the genome of said HIV-1 wherein said deletion mutation results in said genome being unable to synthesize a polypeptide or protein from a pathogenic strain of HIV-1 or directing the synthesis of a truncated form of said polypeptide or protein wherein the presence f a such a mutation is indicative of the presence of a non-pathogenic strain of HIV-1.

A further aspect of the invention contemplates a method for determining the pathogenicity of a strain of HIV-1 after said HIV-1 strain infects cells of an individual, said method comprising contacting a biological sample from said individual with a peptide corresponding to a deleted or truncated region of an HIV-1-derived protein and screening for the absence of antibody binding to said peptide, wherein the absence of antibody binding is indicative of a deletion or truncation in that protein and further indicative of the non-pathogenicity of said strain of HIV-1.

Another aspect of the invention is directed to a method for determining the pathogenicity of a strain of HIV-1 after said HIV-1 strain infects cells of an individual, said method comprising contacting a biological sample from said individual with an effective amount of a peptide having an amino acid sequence comprising or within amino acids 162-177 of wild-type HIV-1_{NL43} Nef, said contact being for a time and under conditions sufficient for an antibody if present in said biological sample to form a complex with said peptide and then detecting the presence of said complex wherein the absence of a complex in an individual seropositive for HIV-1 is indicative of that individual being infected with a non-pathogenic strain of HIV-1.

Still another aspect of the invention provides a method for determining the pathogenicity of an HIV-1 strain after said HIV-1 strain infects cells of an individual, said method comprising determining the presence of a mutation in the genome of said HIV-1 wherein the presence of the mutation results in a Nef protein substantially lacking amino acids 162 to 177 of Nef of a wild-type strain of HIV-1 and wherein said mutation is indicative of a non-pathogenic strain of HIV-1.

Still yet another aspect of the invention is directed to a synthetic peptide comprising a sequence of amino acids defined by SEQ ID NO:801 or a part or fragment thereof.

In yet a further aspect of the invention there is contemplated a method for determining the risk of an individual seropositive for HIV-1 developing symptoms of AIDS, said method comprising contacting antibodies from said patient with a synthetic peptide defined by SEQ ID NO:801 or a part or derivative thereof and detecting non-binding of antibodies to said peptide wherein the substantial absence of antibodies to said peptide is indicative of a low risk of the individual developing AIDS.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a representation showing the alignment of the nucleotide sequences from donor D36 peripheral blood mononuclear cell (PBMC) [D36P] and non-pathogenic HIV-1 from recipient C18 HIV$_{StV}$ [C18S], C18 HIV$_{MBC}$ [C18M] and C98 HIV [C98H] and C54 PBMC [C54P] with the equivalent region of HIV-1$_{NL43}$. Sequences labelled PBMC are from patient PBMC, those labelled HIV are from virus isolated from patient PBMC and grown in culture. Numbering for HIV-1$_{NL43}$ is as per Myer *et al* (1992; 1994) where nucleotide 1 is the first nucleotide of the complete proviral DNA sequence. D36P, C18S, C18M, C98H and C54P are numbered from the start of the region sequenced. Identity with NL43 sequence is shown by (*). Deleted nucleotides are shown by (-). Spaces introduced to maximise alignment are shown by (,). Features in HIV-1$_{NL43}$ are marked by overlining the sequence, features in D36 and C18 are marked by underlining the sequence.

Figure 2 shows the alignment of encoded amino acid sequences of (a) *tat* exon 3 and (b) *rev* exon 3 from HIV-1$_{NL43}$, D36 PBMC, C18 HIV$_{StV}$ and C98 HIV. In-phase termination codons (*) and NL43 encoded amino acid numbers are shown.

Figure 3 is a representation showing the alignment of C-terminal envelope glycoprotein gp41 amino acid sequences encoded by D36 PMBC, C18 HIV$_{StV}$, C18 HIV$_{MBC}$ and C98 HIV. Numbering is that of the amino acid sequence of the mature envelope glycoprotein of HIV-1$_{NL43}$. Termination in shown by (#).

Figure 4 is a representation showing alignment of amino acid sequences encoded by the *nef* genes of HIV-1$_{NL43}$, D36 PBMC, C18 HIV$_{StV}$, C18 HIV$_{MBC}$ and C98 HIV. In phase termination codons are shown by (#). Identical amino acids are shown by (*). Residues underlined are those immediately before a deletion.

Figure 5 shows duplication of NFκB and Sp1 sequences in D36 PBMC, C18 HIV$_{StV}$, C18 HIV$_{MBC}$ and C98 HIV demonstrated by alignment of their low homology region sequences with the NFκB-Sp1 region of HIV-1$_{NL43}$. Nucleotide numbering according

to Figure 1. Identity with NL43 sequence shown by (*) and NFKB and Sp1 sites in NL43 overlined. Position f *nef*/LTR region sequence deletion shown by (▲).

Figure 6 is a graphical representation showing replication of C18 and C98 viral isolates and D36 PBMCs from asymptomatic patients in PHA-stimulated PBMCs.

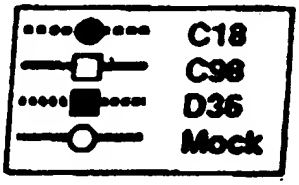
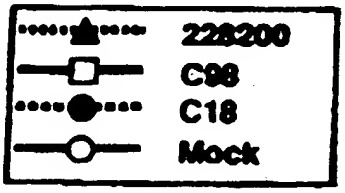


Figure 7 is a graphical representation showing replication of viral isolates from asymptomatic patients in non-PHA stimulated PBMCs.



228200 is an Australian isolate of HIV-1 described by Kiernan, R. *et al* (1990). Its characteristics include being T cell trophic, with fast kinetics, high producer of HIV-1 and/or SI phenotype.

Figure 8 is a graphical representation of cell surface receptor expression for syncytia-inducing (SI)/ non-syncytia-inducing (NSI)/asymptomatic patient isolates.

228200 is defined in the legend to Figure 7. 243925 is a viral isolate of HIV-1 which is monocyte/macrophage trophic and exhibits NSI phenotype (Dr Karen Coats-Fryer, PhD thesis entitled "Viral determinants of HIV-1 syncytium formation", the University f Melbourne, Parkville, Victoria, Australia.

Figure 9 is a representation of the nucleotide sequence of C18 HIV-1_{MBC} (SEQ ID NO: 800).

Figure 10(a) - (g) are graphical representations showing clinical immunology of cohort; (a) CD3; (b)(i) CD4 (ii) CD4%; (c)(i) CD8; (ii) CD8%; (d) lymphocyte count; (e) CD4/CD8 ratio; (f) β-2-microglobulin; and (g) Kaplan-Meier estimates of time to disease progression (Cox & Oakes, 1989).

Figure 11 is a schematic representation of the deletion mutants of the present invention.

Figure 12 shows reactivity of sera from LTP individuals (1a); HIV-1-ve individuals (1bi, 1bii); individuals with autoimmune disease (A/HIV-1) (1biii); LTNP1 (1c) and LTNP2 (1d) with full length Nef 27 derived from HIV-1_{NL43} (referred to herein as "Nef 27"). The term "LTNP" is an abbreviation for "Long Term Non-Progessor". NTNP1 and LTNP2 are defined in Example 16.

Wells of 96-well polystyrene microtitre plates coated with purified Nef 27 (100 ng/well) were incubated with sera (titrated from 1:100 to 1:10,000) obtained from LTP individuals, HIV-1-ve individuals, individuals with autoimmune disease, LTNP1 and LTNP2. The presence of antibodies in the sera which recognise Nef 27 were detected using a biotin-streptavidin HRP system with *o*-phenylenediamine as substrate. Absorbance was measured using a Titertek plate reader at wavelengths of 630 and 450 nm.

Figure 13a shows reactivity of sera from LTP individuals against Nef-derived peptides. Synthetic peptides corresponding to amino acid residues 1 to 19 (i), 20 to 36 (ii), 44 to 65 (iii); 72 to 83 (iv), 89 to 97 (v9); 109 to 114 (vi), 164 to 186 (vii), 187 to 206 (viii), 121 to 135 (ix) and 162 to 177 (x) of HIV-1_{NL43} Nef 27 were coated onto wells of 96-well microtitre plates at a concentration of 500 ng/well. Sera (titrated from 1:300 to 1;100,000) from the LTP individuals were then incubated with the immobilised peptides and the presence of antibodies in the sera which recognise the Nef-derived peptide were detected using a biotin-streptavidin HRP system with *o*-phenylenediamine

as substrate. Absorbance was measured using a Titertek plate reader at wavelengths of 630 and 450 nm.

Figure 13b(i) shows reactivity of sera from HIV-1-ve individuals against Nef-derived peptides. Synthetic peptides corresponding to amino acid residues 1 to 19(i), 20 to 36 (ii), 44 to 65 (iii), 72 to 83 (iv), 89 to 97 (v), 109 to 114 (vi) 164 to 186 (vii), 187 to 206 (viii), 121 to 135 (ix) and 162 to 177 (x) of HIV-1_{NL43} Nef were coated onto wells of 96-well microtitre plates at a concentration of 500 ng/well. Sera (titrated from 1:300 to 1:100,000) from A/HIV-1-ve individuals with autoimmune disease was then incubated with the immobilised peptides and the presence of antibodies in the sera which recognise the Nef-derived peptides were detected using a biotin-streptavidin HRP system with *o*-phenylenediamine as substrate. Absorbance was measured using a Titertek plate reader at wavelengths of 630 and 450 nm.

Figure 13b(ii) shows reactivity of sera from autoimmune A/HIV-1-ve individuals against Nef-derived peptides. Synthetic peptides corresponding to amino acid residues 1 to 19 (i), 20 to 36 (ii), 44 to 65 (iii), 72 to 83 (iv), 89 to 97 (v), 109 to 114 (vi), 164 to 186 (vii), 187 to 206 (viii), 121 to 135 (ix) and 162 to 177 (x) of HIV-1_{NL43} Nef were coated onto wells of 96-well microtitre plates at a concentration of 500 ng/well. Sera (titrated from 1:300 to 1:100,000) from A/HIV-1-ve individuals with autoimmune disease was then incubated with the immobilised peptides and the presence of antibodies in the sera which recognise the Nef-derived peptides were detected using a biotin-streptavidin HRP system with *o*-phenylenediamine as substrate. Absorbance was measured using a Titerek plate reader at wavelengths of 630 and 450 nm.

Figure 13c shows reactivity of sera from LTNP1 individuals against Nef-derived peptides. Synthetic peptides corresponding to amino acid residues 1 to 19 (i), 20 to 36 (ii), 44 to 65 (iii), 72 to 83 (iv), 89 to 97 (v), 109 to 114 (vi), 164 to 186 (vii), 187 to 206 (viii), 121 to 135 (ix) and 162 to 177 (x) of HIV-1_{NL43} Nef were coated onto wells of 96-well microtitre plates at a concentration of 500 ng/well. Sera (titrated from 1:300 to 1:100,000) from the LTNP1 individuals were then incubated with the immobilised peptides and the presence of antibodies in the sera which recognise the Nef-derived

peptides were detected using a biotin-streptavidin HRP system with *o*-phenylenediamine as substrate. Absorbance was measured using a Titertek plate reader at wavelengths of 630 and 450 nm.

Figure 13d shows reactivity of sera from LTNP2 individuals against Nef-derived peptides. Synthetic peptides corresponding to amino acid residues 1 to 19 (i), 20 to 36 (ii), 44 to 65 (iii), 72 to 83 (iv), 89 to 97 (v), 109 to 114 (vi), 164 to 186 (vii), 187 to 206 (viii), 121 to 135 (ix) and 162 to 177 (x) of HIV-1_{NL43} Nef were coated onto wells of 96-well microtitre plates at a concentration of 500 ng/well. Sera (titrated from 1:300 to 1:100,000) from the LTNP2 individuals were then incubated with the immobilised peptides and the presence of antibodies in the sera which recognise the Nef-derived peptides were detected using a biotin-streptavidin HRP system with *o*-phenylenediamine as substrate. Absorbance was measured using a Titertek plate reader at wavelengths of 630 and 450 nm.

A summary of the SEQ ID Nos. used in the subject specification is shown below:

SEQ ID NO:	DESCRIPTION
1	Nucleotide sequence of HIV-1_{NL43} genome
2-613	Decanucleotides of *nef* gene from HIV-1_{NL43}
614	Partial nucleotide sequence of D36 HIV-1 isolate
615	Partial nucleotide sequence of C18 HIV-1_{MBC} isolate
616-625	PCR primers shown in Table 1
626-633	Sequence primers shown in Table 2
634	Amino acid residues 15-27 of HIV-1_{NL43} *nef* protein
635	HIV-1_{NL43} *tat* exons (Figure 2)
636	HIV-1 D36 *tat* exons (Figure 2)
637	HIV-1 C18 *tat* exons (Figure 2)
638	HIV-1_{NL43} *rev* exons (Figure 2)
639	HIV-1 D36 *rev* exons (Figure 2)
640	HIV-1 C18 *rev* exons (Figure 2)
641	HIV-1_{NL43} C-terminal of gp41 (Figure 3)
642	HIV-1 D36 C-terminal of gp41 (Figure 3)
643	HIV-1 C18 C-terminal of gp41 (Figure 3)
644	HIV-1_{NL43} *nef* gene (Figure 4)
645	HIV-1 D36 *nef* gene (Figure 4)
646	HIV-1 C18 *nef* gene (Figure 4)
647	HIV-1_{NL43} NFKB/SP1 sequence (Figure 5)
648	HIV-1 D36 NFKB/SP1 sequence (Figure 5)
649	HIV-1 C18 NFKB/SP1 sequence (Figure 5)
650	Nucleotide sequence of *nef* gene from HIV-1_{NL43}
651	Nucleotide sequence of *env* and *nef* regions of HIV-1_{NL43}
652-799	Decanucleotides of LTR region from HIV-1_{NL43}
800	Nucleotide sequence of C18 HIV-1_{MBC}
801	Amino acids 162 to 177 of wild-type HIV-1_{NL43} Nef
802	Nucleotide sequence encoding amino acids 162 to 177 of wild-type HIV-1_{NL43} Nef
803-841	Decanucleotide deletion of Nef gene covering amino acids 162 to 177

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

One aspect of the present invention contemplates a non-pathogenic isolate of HIV-1 or a component, part, fragment or derivative thereof.

In a related embodiment, there is provided a novel isolate of HIV-1 or a component, part, fragment or derivative thereof wherein said HIV-1 isolate is capable of stimulating in a human or primate subject an immune response such as a humoral immune response to at least one HIV-1 glycoprotein such as but not limited to gp41-45, gp120 and/or gp160 while not substantially reducing in said human or primate subject proliferative responses and cytokine production to a mitogen, alloantigen and/or recall antigen compared to a healthy, non-infected human or primate subject. Preferably, the cytokine is IL-2. Preferably, the mitogen is ConA or PHA and the recall antigen is influenza or tetanus toxoid. Preferably, the HIV-1 isolate is non-pathogenic.

More particularly, the present invention relates to an isolated HIV-1 strain which:

(i) is substantially non-pathogenic in human subjects; and

(ii) carries one or more mutations in its genome resulting in the inability to direct synthesis of at least one pathogenic HIV-1-derived polypeptide or protein.

Even more particularly, the present invention provides an isolated HIV-1 strain which:

(i) is substantially non-pathogenic in human subjects; and

(ii) carries a mutation in the *nef* gene and/or a long terminal repeat (LTR) region or in a functionally equivalent location in the HIV-1 genome.

Still even more particularly, the present invention is directed to an isolated virus which:

(i) has a genome which is capable of hybridising under medium stringency conditions to complementary nucleic acid from a pathogenic strain of HIV-1;

(ii) is substantially non-pathogenic in human subjects;

(iii) carries one or more deletion mutations in a region of its genome corresponding to a *nef* gene in said pathogenic strain of HIV-1; and

(iv) optionally carries a mutation in ne or both LTR regions.

In a related embodiment, there is provided an isolated virus which:

(i) has a genome which is capable of hybridising under medium stringency conditions to complementary nucleic acid from a pathogenic strain of HIV-1;

(ii) is substantially non-pathogenic in human subjects;

(iii) carries one or more deletion mutations in an LTR region of its genome; and

(iv) optionally carries a mutation in a region corresponding to a *nef* gene in said pathogenic strain of HIV-1..

In a further related embodiment, there is provided an isolated virus which:

(i) has a genome which is capable of hybridising under medium stringency conditions to complementary nucleic acid from a pathogenic strain of HIV-1;

(ii) is substantially non-pathogenic in human subjects; and

(iii) carries one or more deletion mutations in a region of its genome corresponding to a region which contains *nef* coding sequences and LTR nucleotide sequences.

Another aspect of the present invention is directed to an isolated strain of HIV-1 or a biological source thereof, wherein said HIV-1 has the following characteristics:

(i) is substantially non-pathogenic in human subjects; and

(ii) carries a modified *nef* gene which encodes a *nef* gene product substantially immunologically non-interactive with antibodies to amino acids 162 to 177 f Nef in wild-type HIV-1.

Amino acids of 162-177 of wild-type HIV-1_{NL43} strain (Myers *et al*, 1994) [hereinafter referred to as "HIV-1_{NL43}"] are as follows:

Thr Ser Leu Leu His Pro Val Ser Leu His Gly Met Asp Asp Pro Glu [SEQ ID NO:801].

This aspect of the present invention relates in part to amino acid sequence SEQ ID NO:801 from HIV-1_{NL43} or from the functionally equivalent region of other pathogenic strains of HIV-1.

A further aspect of the present invention contemplates an isolated strain of HIV-1 or a biological source thereof, wherein said HIV-1 has the following characteristics:

(i) is substantially non-pathogenic in human subjects; and

(ii) encodes an Nef protein or portion thereof which is interactive with wild-type HIV-1 Nef antibodies but which is substantially non-interactive with antibodies to amino acids 162 to 177 of wild-type HIV-1 Nef protein.

Still another aspect of the present invention relates to an isolated strain of HIV-1 or a biological source thereof which is substantially non-pathogenic in human subjects and which is substantially incapable of directing synthesis of a Nef protein or portion thereof comprising amino acids 162 to 177 of wild-type HIV-1 Nef protein.

In still yet another aspect of the present invention, there is provided an isolated strain of HIV-1 or a biological source thereof, said HIV-1 being substantially non-pathogenic in humans and comprising a mutation in its genome corresponding to amino acids 162 to 177 of wild-type HIV-1 Nef such that these amino acids are substantially not represented in a Nef protein or derivative thereof produced by said isolated HIV-1 strain, or insufficient of the amino acid sequence is represented to induce an immune response to that region of Nef.

In a related embodiment, the genomic mutation in the non-pathogenic strain of HIV-1 is a mutation in one or more of nucleotides 9271 to 9317 relative to HIV-1_{NL43} or in a functionally equivalent region in another pathogenic strain of HIV-1.

In a related embodiment, there is provided a non-pathogenic strain of HIV-1 comprising a genome which is substantially incapable of hybridising under medium stringent conditions a nucleic acid molecule comprising that sequence of nucleotides which encodes all or part of amino acids 162 to 177 of wild-type HIV-1. Preferably, the nucleic acid molecule is a synthetic oligonucleotide.

In a particularly preferred embodiment, the present invention provides non-pathogenic HIV-1 isolate C18 deposited at the ECACC on 17 October, 1994 under Provisional Accession Number V94101706.

In a related embodiment, the present invention provides non-pathogenic HIV-1 isolate C98 deposited at the ECACC on 31 October, 1994 under Provisional Accession Number V941031169.

In another embodiment, the present invention provides non-pathogenic HIV-1 isolate C54 deposited at ECACC on 10 March, 1995 under Provisional Accession N . V95031022.

Although pathogenicity is a relative term, it is used herein in relation to the capacity f a strain of HIV-1 to induce AIDS or AIDS-related disorders in an individual over time. Accordingly, a "non-pathogenic" strain of HIV-1 is a strain which, at the clinical level, does not lead to the development of AIDS, at least within the median time of 6-10 years following infection with HIV-1. At the laboratory level, a non-pathogenic strain of HIV-1 is considered not to alter CD4+ cell counts or β_2-microglobulin concentrations. In addition, a non-pathogenic strain of HIV-1 may not alter CD8+ and CD3+ cell counts and would not alter lymphocyte counts. CD4+:CD8+ ratios also remain unchanged relative to normal non-infected individuals. Furthermore, generally, a non-pathogenic strain of HIV-1 does not induce p24 antigenaemia. A non-pathogenic HIV-1 of the present invention is generally still infectious but individuals infected with the virus remain free of symptoms for at least 6-10 years after infection.

A laboratory classified non-pathogenic strain of HIV-1 may be determined at any time after infection. The term "non-pathogenic" is not to be considered as a strain that is never pathogenic under any conditions as this might depend on the host individual, the level of immune responsiveness in that individual and the extent or otherwise of other, for example, immune comprising disorders. Accordingly, a "non-pathogenic" HIV-1 isolate of the present inventi n may also be considered a "low virulent" strain of the

virus. A non-pathogenic strain of HIV-1 as contemplated herein may be isolated from an asymptomatic individual or may be derived from a pathogenic strain by mutation. Although the present invention is not to be limited to any particular pathogenic strain of HIV-1, for reference purposes, an example of a pathogenic strain is HIV-1_{NL43} strain as described by Myers *et al* (1992; 1994).

The non-pathogenic nature of the HIV-1 of the present invention is conveniently evidenced by the cohort of seven individuals comprising one donor and six recipients which have remained free of symptoms or signs of HIV-1 infection for greater than the median time of 6-10 years. However, the individuals of the cohort are seropositive for HIV-1 following infection with the virus as determined by Western blot analysis and genetic analysis (e.g. using PCR techniques). A seropositive individual is one showing reactivity to at least one HIV-1 glycoprotein (such as but not limited to gp 41-45, gp120, gp160) and at least three other virus-specific bands.

In accordance with the present invention, a non-pathogenic HIV-1 isolate is also a strain of HIV-1 which:

(i) induces an immune response in a human or primate subject; and

(ii) does not substantially reduce proliferative responses or cytokine production to a mitogen, alloantigen and/or recall antigen relative to a healthy, non-infected subject.

Preferably, the immune response such as to a glycoprotein, for example gp41-45, gp120 and/or gp160. Preferably, the cytokine monitored is an interleukin, such as IL-2. Preferably, the recall antigen is influenza or tetanus toxoid. A non-pathogenic HIV-1 isolate is also one which:

(iii) does not substantially alter proliferative responses or cytokine production to allorgeneic mononuclear cells.

Furthermore, a non-pathogenic strain of HIV-1 carries a deletion in an HIV-1-derived protein which results in an altered immunological profile. In a particularly preferred embodiment, the non-pathogenic strain f HIV-1 is substantially incapable of inducing

an antibody response to amino acids 162 to 177 to wild-type HIV-1 Nef protein. According to this preferred aspect of the present invention, there is provided a non-pathogenic HIV-1 isolate which:

(i) induces an immune response in a human or primate subject;

(ii) does not substantially reduce proliferative responses or cytokine production to a mitogen, alloantigen and/or recall antigen relative to a healthy, non-infected subject; and

(iii) is substantially incapable of inducing an antibody response to amino acids 162 to 177 of wild-type HIV-1 Nef protein.

The genomes or complementary DNA therefrom of the non-pathogenic HIV-1 isolates of the present invention are capable of hybridising under medium stringency conditions to the corresponding genome or complementary DNA of a pathogenic strain of HIV-1 (e.g. HIV-1 strain HIV-1_{NL43}). The ability to hybridize to a pathogenic strain of HIV-1 only applies to a comparison of the entire genome/complementary DNA of a non-pathogenic strain or a fragment which includes genetic material corresponding to a region in the genome 3′ of the *nef* gene in a pathogenic strain of HIV-1.

Reference herein to "wild-type HIV-1" is meant to include reference to architypal pathogenic strain HIV-1_{NL43} (Myers *et al*, 1992; 1994). For the purposes of reference only, a suitable genomic nucleotide sequence from HIV-1_{NL43} is set forth in SEQ ID NO: 1 (Myers *et al*, 1992; 1994):

```
   1 TGGAAGGGCTAATTTGGTCCCAAAAAAGACAAGAGATCCTTGATCTGTGG
  51 ATCTACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGG
 101 GCCAGGGATCAGATATCCACTGACCTTTGGATGGTGCTTCAAGTTAGTAC
 151 CAGTTGAACCAGAGCAAGTAGAAGAGGCCAAATAAGGAGAGAAGAACAGC
 201 TTGTTACACCCTATGAGCCAGCATGGGATGGAGGACCCGGAGGGAGAAGT
 251 ATTAGTGTGGAAGTTTGACAGCCTCCTAGCATTTCGTCACATGGCCCGAG
 301 AGCTGCATCCGGAGTACTACAAAGACTGCTGACATCGAGCTTTCTACAAG
 351 GGACTTTCCGCTGGGGACTTTCCAGGGAGGTGTGGCCTGGGCGGGACTGG
 401 GGAGTGGCGAGCCCTCAGATGCTACATATAAGCAGCTGCTTTTTGCCTGT
 451 ACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTA
 501 ACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTCA
 551 AAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTC
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601 AGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGG
651 GACTTGAAAGCGAAAGTAAAGCCAGAGGAGATCTCTCGACGCAGGACTCG
701 GCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTA
751 CGCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAG
801 AGCGTCGGTATTAAGCGGGGGAGAATTAGATAAATGGGAAAAAATTCGGT
851 TAAGGCCAGGGGGAAAGAAACAATATAAACTAAAACATATAGTATGGGCA
901 AGCAGGGAGCTAGAACGATTCGCAGTTAATCCTGGCCTTTTAGAGACATC
951 AGAAGGCTGTAGACAAATACTGGGACAGCTACAACCATCCCTTCAGACAG
1001 GATCAGAAGAACTTAGATCATTATATAATACAATAGCAGTCCTCTATTGT
1051 GTGCATCAAAGGATAGATGTAAAAGACACCAAGGAAGCCTTAGATAAGAT
1101 AGAGGAAGAGCAAAACAAAAGTAAGAAAAAGGCACAGCAAGCAGCAGCTG
1151 ACACAGGAAACAACAGCCAGGTCAGCCAAAATTACCCTATAGTGCAGAAC
1201 CTCCAGGGGCAAATGGTACATCAGGCCATATCACCTAGAACTTTAAATGC
1251 ATGGGTAAAAGTAGTAGAAGAGAAGGCTTTCAGCCCAGAAGTAATACCCA
1301 TGTTTTCAGCATTATCAGAAGGAGCCACCCCACAAGATTTAAATACCATG
1351 CTAAACACAGTGGGGGGACATCAAGCAGCCATGCAAATGTTAAAAGAGAC
1401 CATCAATGAGGAAGCTGCAGAATGGGATAGATTGCATCCAGTGCATGCAG
1451 GGCCTATTGCACCAGGCCAGATGAGAGAACCAAGGGGAAGTGACATAGCA
1501 GGAACTACTAGTACCCTTCAGGAACAAATAGGATGGATGACACATAATCC
1551 ACCTATCCCAGTAGGAGAAATCTATAAAAGATGGATAATCCTGGGATTAA
1601 ATAAAATAGTAAGAATGTATAGCCCTACCAGCATTCTGGACATAAGACAA
1651 GGACCAAAGGAACCCTTTAGAGACTATGTAGACCGATTCTATAAAACTCT
1701 AAGAGCCGAGCAAGCTTCACAAGAGGTAAAAAATTGGATGACAGAAACCT
1751 TGTTGGTCCAAAATGCGAACCCAGATTGTAAGACTATTTTAAAAGCATTG
1801 GGACCAGGAGCGACACTAGAAGAAATGATGACAGCATGTCAGGGAGTGGG
1851 GGGACCCGGCCATAAAGCAAGAGTTTTGGCTGAAGCAATGAGCCAAGTAA
1901 CAAATCCAGCTACCATAATGATACAGAAAGGCAATTTTAGGAACCAAAGA
1951 AAGACTGTTAAGTGTTTCAATTGTGGCAAAGAAGGGCACATAGCCAAAAA
2001 TTGCAGGGCCCCTAGGAAAAAGGGCTGTTGGAAATGTGGAAAGGAAGGAC
2051 ACCAAATGAAAGATTGTACTGAGAGACAGGCTAATTTTTTAGGGAAGATC
2101 TGGCCTTCCCACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGA
2151 GCCAACAGCCCCACCAGAAGAGAGCTTCAGGTTTGGGGAAGAGACAACAA
2201 CTCCCTCTCAGAAGCAGGAGCCGATAGACAAGGAACTGTATCCTTTAGCT
2251 TCCCTCAGATCACTCTTTGGCAGCGACCCCTCGTCACAATAAAGATAGGG
2301 GGGCAATTAAAGGAAGCTCTATTAGATACAGGAGCAGATGATACAGTATT
2351 AGAAGAAATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAA
2401 TTGGAGGTTTTATCAAAGTAGGACAGTATGATCAGATACTCATAGAAATC
2451 TGCGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAA

2501 CATAATTGGAAGAAATCTGTTGACTCAGATTGGCTGCACTTTAAATTTTC
2551 CCATTAGTCCTATTGAGACTGTACCAGTAAAATTAAAGCCAGGAATGGAT
2601 GGCCCAAAAGTTAAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATT
2651 AGTAGAAATTTGTACAGAAATGGAAAAGGAAGGAAAAATTTCAAAAATTG
2701 GGCCTGAAAATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAAGAC
2751 AGTACTAAATGGAGAAAATTAGTAGATTTCAGAGAACTTAATAAGAGAAC
2801 TCAAGATTTCTGGGAAGTTCAATTAGGAATACCACATCCTGCAGGGTTAA
2851 AACAGAAAAAATCAGTAACAGTACTGGATGTGGGCGATGCATATTTTTCA
2901 GTTCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTACCATACCTAG
2951 TATAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCAC
3001 AGGGATGGAAAGGATCACCAGCAATATTCCAGTGTAGCATGACAAAAATC
3051 TTAGAGCCTTTTAGAAAACAAAATCCAGACATAGTCATCTATCAATACAT
3101 GGATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAA
3151 AAATAGAGGAACTGAGACAACATCTGTTGAGGTGGGGATTTACCACACCA
3201 GACAAAAAACATCAGAAAGAACCTCCATTCCTTTGGATGGGTTATGAACT
3251 CCATCCTGATAAATGGACAGTACAGCCTATAGTGCTGCCAGAAAAGGACA
3301 GCTGGACTGTCAATGACATACAGAAATTAGTGGGAAAATTGAATTGGGCA
3351 AGTCAGATTTATGCAGGGATTAAAGTAAGGCAATTATGTAAACTTCTTAG
3401 GGGAACCAAAGCACTAACAGAAGTAGTACCACTAACAGAAGAAGCAGAGC
3451 TAGAACTGGCAGAAAACAGGGAGATTCTAAAAGAACCGGTACATGGAGTG
3501 TATTATGACCCATCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCA
3551 AGGCCAATGGACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAA
3601 CAGGAAAATATGCAAGAATGAAGGGTGCCCACACTAATGATGTGAAACAA
3651 TTAACAGAGGCAGTACAAAAAATAGCCACAGAAAGCATAGTAATATGGGG
3701 AAAGACTCCTAAATTTAAATTACCCATACAAAAGGAAACATGGGAAGCAT
3751 GGTGGACAGAGTATTGGCAAGCCACCTGGATTCCTGAGTGGGAGTTTGTC
3801 AATACCCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGAAAGAACCCAT
3851 AATAGGAGCAGAAACTTTCTATGTAGATGGGGCAGCCAATAGGGAAACTA
3901 AATTAGGAAAAGCAGGATATGTAACTGACAGAGGAAGACAAAAAGTTGTC
3951 CCCCTAACGGACACAACAAATCAGAAGACTGAGTTACAAGCAATTCATCT
4001 AGCTTTGCAGGATTCGGGATTAGAAGTAAACATAGTGACAGACTCACAAT
4051 ATGCATTGGGAATCATTCAAGCACAACCAGATAAGAGTGAATCAGAGTTA
4101 GTCAGTCAAATAATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGC
4151 ATGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACAAGTAGATGGGT
4201 TGGTCAGTGCTGGAATCAGGAAAGTACTATTTTTAGATGGAATAGATAAG
4251 GCCCAAGAAGAACATGAGAAATATCACAGTAATTGGAGAGCAATGGCTAG
4301 TGATTTTAACCTACCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTG
4351 ATAAATGTCAGCTAAAAGGGGAAGCCATGCATGGACAAGTAGACTGTAGC

4401 CCAGGAATATGGCAGCTAGATTGTACACATTTAGAAGGAAAAGTTATCTT
4451 GGTAGCAGTTCATGTAGCCAGTGGATATATAGAAGCAGAAGTAATTCCAG
4501 CAGAGACAGGGCAAGAAACAGCATACTTCCTCTTAAAATTAGCAGGAAGA
4551 TGGCCAGTAAAAACAGTACATACAGACAATGGCAGCAATTTCACCAGTAC
4601 TACAGTTAAGGCCGCCTGTTGGTGGGCGGGGATCAAGCAGGAATTTGGCA
4651 TTCCCTACAATCCCCAAAGTCAAGGAGTAATAGAATCTATGAATAAAGAA
4701 TTAAAGAAAATTATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGAC
4751 AGCAGTACAAATGGCAGTATTCATCCACAATTTTAAAAGAAAAGGGGGGA
4801 TTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGAC
4851 ATACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCG
4901 GGTTTATTACAGGGACAGCAGAGATCCAGTTTGGAAAGGACCAGCAAAGC
4951 TCCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGATAATAGTGACATA
5001 AAAGTAGTGCCAAGAAGAAAAGCAAAGATCATCAGGGATTATGGAAAACA
5051 GATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAACACA
5101 TGGAAAAGATTAGTAAAACACCATATGTATATTTCAAGGAAAGCTAAGGA
5151 CTGGTTTTATAGACATCACTATGAAAGTACTAATCCAAAAATAAGTTCAG
5201 AAGTACACATCCCACTAGGGGATGCTAAATTAGTAATAACAACATATTGG
5251 GGTCTGCATACAGGAGAAAGAGACTGGCATTTGGGTCAGGGAGTCTCCAT
5301 AGAATGGAGGAAAAAGAGATATAGCACACAAGTAGACCCTGACCTAGCAG
5351 ACCAACTAATTCATCTGCACTATTTTGATTGTTTTTCAGAATCTGCTATA
5401 AGAAATACCATATTAGGACGTATAGTTAGTCCTAGGTGTGAATATCAAGC
5451 AGGACATAACAAGGTAGGATCTCTACAGTACTTGGCACTAGCAGCATTAA
5501 TAAAACCAAAACAGATAAAGCCACCTTTGCCTAGTGTTAGGAAACTGACA
5551 GAGGACAGATGGAACAAGCCCCAGAAGACCAAGGGCCACAGAGGGAGCCA
5601 TACAATGAATGGACACTAGAGCTTTTAGAGGAACTTAAGAGTGAAGCTGT
5651 TAGACATTTTCCTAGGATATGGCTCCATAACTTAGGACAACATATCTATG
5701 AAACTTACGGGGATACTTGGGCAGGAGTGGAAGCCATAATAAGAATTCTG
5751 CAACAACTGCTGTTTATCCATTTCAGAATTGGGTGTCGACATAGCAGAAT
5801 AGGCGTTACTCGACAGAGGAGAGCAAGAAATGGAGCCAGTAGATCCTAGA
5851 CTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAACTGCTTGTACCAA
5901 TTGCTATTGTAAAAAGTGTTGCTTTCATTGCCAAGTTTGTTTCATGACAA
5951 AAGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGA
6001 GCTCATCAGAACAGTCAGACTCATCAAGCTTCTCTATCAAAGCAGTAAGT
6051 AGTACATGTAATGCAACCTATAATAGTAGCAATAGTAGCATTAGTAGTAG
6101 CAATAATAATAGCAATAGTTGTGTGGTCCATAGTAATCATAGAATATAGG
6151 AAAATATTAAGACAAAGAAAAATAGACAGGTTAATTGATAGACTAATAGA
6201 AAGAGCAGAAGACAGTGGCAATGAGAGTGAAGGAGAAGTATCAGCACTTG
6251 TGGAGATGGGGGTGGAAATGGGGCACCATGCTCCTTGGGATATTGATGAT

6301 CTGTAGTGCTACAGAAAAATTGTGGGTCACAGTCTATTATGGGGTACCTG
6351 TGTGGAAGGAAGCAACCACCACTCTATTTTGTGCATCAGATGCTAAAGCA
6401 TATGATACAGAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCAC
6451 AGACCCCAACCCACAAGAAGTAGTATTGGTAAATGTGACAGAAAATTTTA
6501 ACATGTGGAAAAATGACATGGTAGAACAGATGCATGAGGATATAATCAGT
6551 TTATGGGATCAAAGCCTAAAGCCATGTGTAAAATTAACCCCACTCTGTGT
6601 TAGTTTAAAGTGCACTGATTTGAAGAATGATACTAATACCAATAGTAGTA
6651 GCGGGAGAATGATAATGGAGAAAGGAGAGATAAAAAACTGCTCTTTCAAT
6701 ATCAGCACAAGCATAAGAGATAAGGTGCAGAAAGAATATGCATTCTTTTA
6751 TAAACTTGATATAGTACCAATAGATAATACCAGCTATAGGTTGATAAGTT
6801 GTAACACCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTTGAGCCA
6851 ATTCCCATACATTATTGTGCCCCGGCTGGTTTTGCGATTCTAAAATGTAA
6901 TAATAAGACGTTCAATGGAACAGGACCATGTACAAATGTCAGCACAGTAC
6951 AATGTACACATGGAATCAGGCCAGTAGTATCAACTCAACTGCTGTTAAAT
7001 GGCAGTCTAGCAGAAGAAGATGTAGTAATTAGATCTGCCAATTTCACAGA
7051 CAATGCTAAAACCATAATAGTACAGCTGAACACATCTGTAGAAATTAATT
7101 GTACAAGACCCAACAACAATACAAGAAAAAGTATCCGTATCCAGAGGGGA
7151 CCAGGGAGAGCATTTGTTACAATAGGAAAAATAGGAAATATGAGACAAGC
7201 ACATTGTAACATTAGTAGAGCAAAATGGAATGCCACTTTAAAACAGATAG
7251 CTAGCAAATTAAGAGAACAATTTGGAAATAATAAAACAATAATCTTTAAG
7301 CAATCCTCAGGAGGGGACCCAGAAATTGTAACGCACAGTTTTAATTGTGG
7351 AGGGGAATTTTTCTACTGTAATTCAACACAACTGTTTAATAGTACTTGGT
7401 TTAATAGTACTTGGAGTACTGAAGGGTCAAATAACACTGAAGGAAGTGAC
7451 ACAATCACACTCCCATGCAGAATAAAACAATTTATAAACATGTGGCAGGA
7501 AGTAGGAAAAGCAATGTATGCCCCTCCCATCAGTGGACAAATTAGATGTT
7551 CATCAAATATTACTGGGCTGCTATTAACAAGAGATGGTGGTAATAACAAC
7601 AATGGGTCCGAGATCTTCAGACCTGGAGGAGGCGATATGAGGGACAATTG
7651 GAGAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAG
7701 TAGCACCCACCAAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCA
7751 GTGGGAATAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCAC
7801 TATGGGCTGCACGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGT
7851 CTGATATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAA
7901 CAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAACAGCTCCAGGCAAG
7951 AATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTT
8001 GGGGTTGCTCTGGAAAACTCATTTGCACCACTGCTGTGCCTTGGAATGCT
8051 AGTTGGAGTAATAAATCTCTGGAACAGATTTGGAATAACATGACCTGGAT
8101 GGAGTGGGACAGAGAAATTAACAATTACACAAGCTTAATACACTCCTTAA
8151 TTGAAGAATCGCAAAACCAGCAAGAAAAGAATGAACAAGAATTATTGGAA

8201 TTAGATAAATGGGCAAGTTTGTGGAATTGGTTTAACATAACAAATTGGCT
8251 GTGGTATATAAAATTATTCATAATGATAGTAGGAGGCTTGGTAGGTTTAA
8301 GAATAGTTTTTGCTGTACTTTCTATAGTGAATAGAGTTAGGCAGGGATAT
8351 TCACCATTATCGTTTCAGACCCACCTCCCAATCCCGAGGGGACCCGACAG
8401 GCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCA
8451 TTCGATTAGTGAACGGATCCTTAGCACTTATCTGGGACGATCTGCGGAGC
8501 CTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACTCTTGATTGTAAC
8551 GAGGATTGTGGAACTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATT
8601 GGTGGAATCTCCTACAGTATTGGAGTCAGGAACTAAAGAATAGTGCTGTT
8651 AACTTGCTCAATGCCACAGCCATAGCAGTAGCTGAGGGGACAGATAGGGT
8701 TATAGAAGTATTACAAGCAGCTTATAGAGCTATTCGCCACATACCTAGAA
8751 GAATAAGACAGGGCTTGGAAAGGATTTTGCTATAAGATGGGTGGCAAGTG
8801 GTCAAAAAGTAGTGTGATTGGATGGCCTGCTGTAAGGGAAAGAATGAGAC
8851 GAGCTGAGCCAGCAGCAGATGGGGTGGGAGCAGTATCTCGAGACCTAGAA
8901 AAACATGGAGCAATCACAAGTAGCAATACAGCAGCTAACAATGCTGCTTG
8951 TGCCTGGCTAGAAGCACAAGAGGAGGAAGAGGTGGGTTTTCCAGTCACAC
9001 CTCAGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGC
9051 CACTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACTCCCAAAG
9101 AAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCC
9151 CTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGACC
9201 TTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGA
9251 GGCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATG
9301 GAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGC
9351 CTAGCATTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAA
9401 CTGCTGACATCGAGCTTGCTACAAGGGACTTTCCGCTGGGGACTTTCCAG
9451 GGAGGCGTGGCCTGGGCGGGACTGGGGAGTGGCGAGCCCTCAGATGCTGC
9501 ATATAAGCAGCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGA
9551 TCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCT
9601 CAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTG
9651 TGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAA
9701 TCTCTAGCA

However, for the purposes of comparing the nucleotide sequences of non-pathogenic HIV-1 strains including the ability to hybridize to a reference strain, the present invention extends to a genomic nucleotide sequence from any pathogenic strain of HIV-1.

Reference to a biological source includes blood or blood-related products or components such as lymphocytes, plasma, tissue fluid and tissue extracts.

Accordingly, in a particularly preferred embodiment, there is provided a viral isolate which:

(i) carries a genome which is capable of hybridising under medium stringency conditions to SEQ ID NO: 1 or a complementary form thereof or an analogous sequence from another pathogenic strain of HIV-1; and

(ii) carries a deletion mutation in a region corresponding to the *nef* gene and/or in an LTR region. Generally, such an HIV-1 isolate is non-pathogenic as hereinbefore defined.

In a related embodiment, there is provided an isolated virus which:

(i) has a genome which is capable of hybridising under medium stringency conditions to complementary nucleic acid from a pathogenic strain of HIV-1; and

(ii) carries one or more deletion mutations in a region of its genome corresponding to a region which contains *nef* coding sequences and LTR nucleotide sequences.

For the purposes of defining the level of stringency, reference can conveniently be made to Maniatis *et al* (1982) at pages 387-389 which is herein incorporated by reference where the washing steps disclosed are considered high stringency. A low stringency is defined herein as being in 1-3X SSC/0.1-0.5% w/v SDS at 37-50°C for 2-3 hours. Depending on the source and concentration of nucleic acid involved in the hybridisation, alternative conditions of stringency may be employed such as medium stringent conditions which are considered herein to be 0.1-1X SSC/0.25-0.5% w/v SDS at ≥ 45°C for 2-3 hours or high stringent conditions considered herein to be 0.1-1X SSC/0.1% w/v SDS at 60°C for 1-3 hours.

In a particularly preferred embodiment of the present invention, the non-pathogenic strain of HIV-1 carries a mutati n in the *nef* gene and/or LTR region of the genome.

In an even more preferred embodiment, the non-pathogenic strain f HIV-1 carries a mutation in the *nef* gene such that a Nef protein is not produced or a modified Nef protein is produced substantially not carrying amino acids 162 to 177 or a portion thereof from wild-type Nef.

A "mutation" is considered herein to include a single or multiple nucleotide substitution, deletion and/or addition. Most preferred mutations are single or multiple deletions f at least one, most preferably at least ten and even more preferably at least twenty contiguous nucleotides from a region corresponding to the *nef* gene and/or the LTR region. When the non-pathogenic virus carries a mutation in the LTR region, this generally occurs 5' of the Sp1 sites. A particularly preferred deletion is from a region within the *nef* gene corresponding to amino acids 162 to 177 of the Nef protein.

According to a preferred aspect of the present invention, there is provided a viral isolate which:

(i) is reactive to antibodies to a glycoprotein from HIV-1 such as at least one of gp41-45, gp120 and/or gp160;

(ii) is substantially non-pathogenic in human subjects; and

(iii) carries a deletion mutation of at least ten nucleotides in a region corresponding to the *nef* gene and/or LTR region of a pathogenic strain of HIV-1.

In another embodiment, there is provided a viral isolate which:

(i) is capable of inducing an immune response to at least one of *gag*, *pol* and/or *env*;

(ii) is substantially non-pathogenic in human subjects; and

(iii) carries a deletion mutation of at least ten nucleotides in a region corresponding to the *nef* gene and/or LTR region of a pathogenic strain of HIV-1.

Preferably, in respect of the latter embodiment, the immune response is an antibody or a cell mediated response. In a most preferred embodiment, the immune response is a humoral immune response.

In another aspect of the present invention, there is provided a viral isolate which:

(i) is interactive to antibodies to a glycoprotein from HIV-1 such as at least one f gp41-45, gp120 and/or gp160;

(ii) is substantially non-pathogenic in human subjects; and

(iii) carries a deletion mutation of at least ten nucleotides in a region corresponding to all or part of amino acids 162 to 177 encoded by the *nef* gene of a pathogenic strain of HIV-1.

The nucleotide sequence of the *nef* gene in HIV-1_{NL43} is defined in SEQ ID NO: 650:

ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGATTGGATGGCCTGCTGTAAGGGAAAGAAT
GAGACGAGCTGAGCCAGCAGCAGATGGGGTGGGAGCAGTATCTCGAGACCTAGAAAAAC
ATGGAGCAATCACAAGTAGCAATACAGCAGCTAACAATGCTGCTTGTGCCTGGCTAGAA
GCACAAGAGGAGGAAGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAAT
GACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAAG
GGCTAATTCACTCCCAAAGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAA
GGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGAC
CTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATA
AAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGATGACCCTGAG
AGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTCATCACGTGGCCCGAGA
GCTGCATCCGGAGTACTTCAAGAACTGCTGA

The nucleotide sequence encoding amino acids 162-177 of wild-type HIV-1_{NL43} Nef is as follows:

ACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGATGACCCTGAG [SEQ ID NO:802].

The present invention extends to any or all single or multiple nucleotide deletions to a contiguous series of at least ten nucleotides from the *nef* gene which render the strain avirulent. The deletions may encompass the entire gene or parts thereof and may represent a single deletion or two or more deletions. Put in alternative terms, the non-pathogenic HIV-1 isolates of the present invention comprise a nucleotide sequence at the corresponding *nef* gene region non-identifiable to SEQ ID NO: 650, said non-identity comprising at least 5%, more preferably at least 10% and even more preferably at least

20% variation thereon. The present invention particularly extends to any or all single or multiple nucleotide deletions to a contiguous series of at least ten nucleotides from the region of the *nef* gene corresponding to all or part of amino acids 162 to 177 and which render the strain avirulent. Although this aspect of the present invention is exemplified by specific reference to amino acids 162 to 177, the present invention also extends to a functionally analogous sequence where, for example, amino acids are substituted by structurally or functionally similar amino acids. In addition, the present invention extends to any deletion or other mutation in an HIV-1 derived protein which renders that strain non-pathogenic.

In a preferred embodiment, therefore, the present invention contemplates a viral isolate which:

(i) is reactive to antibodies to a glycoprotein from HIV-1 such as at least one of gp41-45, gp120 and/or gp160;

(ii) carries a genome or a part or fragment thereof capable of hybridising under medium stringency conditions to a nucleotide sequence as set forth in SEQ ID NO: 1 or a complementary form thereof or an analogous sequence from another pathogenic strain of HIV-1;

(iii) carries a deletion of at least ten nucleotides in a region corresponding to the *nef* gene in HIV-1_{NL43}; and

wherein said deletion encompasses one or more of the following decanucleotides from the *nef* gene of HIV-1_{NL43} or corresponding sequences from another pathogenic strain of HIV-1:

ATGGGTGGCA(SEQ ID NO: 2); TGGGTGGCAA(SEQ ID NO: 3);
GGGTGGCAAG(SEQ ID NO: 4); GGTGGCAAGT(SEQ ID NO: 5);
GTGGCAAGTG(SEQ ID NO: 6); TGGCAAGTGG(SEQ ID NO: 7);
GGCAAGTGGT(SEQ ID NO: 8); GCAAGTGGTC(SEQ ID NO: 9);
CAAGTGGTCA(SEQ ID NO: 10); AAGTGGTCAA(SEQ ID NO: 11);
AGTGGTCAAA(SEQ ID NO: 12); GTGGTCAAAA(SEQ ID NO: 13);
TGGTCAAAAA(SEQ ID NO: 14); GGTCAAAAAG(SEQ ID NO: 15);
GTCAAAAAGT(SEQ ID NO: 16); TCAAAAAGTA(SEQ ID NO: 17);
CAAAAAGTAG(SEQ ID NO: 18); AAAAAGTAGT(SEQ ID NO: 19);
AAAAGTAGTG(SEQ ID NO: 20); AAAGTAGTGT(SEQ ID NO: 21);
AAGTAGTGTG(SEQ ID NO: 22); AGTAGTGTGA(SEQ ID NO: 23);

GTAGTGTGAT (SEQ ID NO: 24); TAGTGTGATT (SEQ ID NO: 25);
AGTGTGATTG (SEQ ID NO: 26); GTGTGATTGG (SEQ ID NO: 27);
TGTGATTGGA (SEQ ID NO: 28); GTGATTGGAT (SEQ ID NO: 29);
TGATTGGATG (SEQ ID NO: 30); GATTGGATGG (SEQ ID NO: 31);
ATTGGATGGC (SEQ ID NO: 32); TTGGATGGCC (SEQ ID NO: 33);
TGGATGGCCT (SEQ ID NO: 34); GGATGGCCTG (SEQ ID NO: 35);
GATGGCCTGC (SEQ ID NO: 36); ATGGCCTGCT (SEQ ID NO: 37);
TGGCCTGCTG (SEQ ID NO: 38); GGCCTGCTGT (SEQ ID NO: 39);
GCCTGCTGTA (SEQ ID NO: 40); CCTGCTGTAA (SEQ ID NO: 41);
CTGCTGTAAG (SEQ ID NO: 42); TGCTGTAAGG (SEQ ID NO: 43);
GCTGTAAGGG (SEQ ID NO: 44); CTGTAAGGGA (SEQ ID NO: 45);
TGTAAGGGAA (SEQ ID NO: 46); GTAAGGGAAA (SEQ ID NO: 47);
TAAGGGAAAG (SEQ ID NO: 48); AAGGGAAAGA (SEQ ID NO: 49);
AGGGAAAGAA (SEQ ID NO: 50); GGGAAAGAAT (SEQ ID NO: 51);
GGAAAGAATG (SEQ ID NO: 52); GAAAGAATGA (SEQ ID NO: 53);
AAAGAATGAG (SEQ ID NO: 54); AAGAATGAGA (SEQ ID NO: 55);
AGAATGAGAC (SEQ ID NO: 56); GAATGAGACG (SEQ ID NO: 57);
AATGAGACGA (SEQ ID NO: 58); ATGAGACGAG (SEQ ID NO: 59);
TGAGACGAGC (SEQ ID NO: 60); GAGACGAGCT (SEQ ID NO: 61);
AGACGAGCTG (SEQ ID NO: 62); GACGAGCTGA (SEQ ID NO: 63);
ACGAGCTGAG (SEQ ID NO: 64); CGAGCTGAGC (SEQ ID NO: 65);
GAGCTGAGCC (SEQ ID NO: 66); AGCTGAGCCA (SEQ ID NO: 67);
GCTGAGCCAG (SEQ ID NO: 68); CTGAGCCAGC (SEQ ID NO: 69);
TGAGCCAGCA (SEQ ID NO: 70); GAGCCAGCAG (SEQ ID NO: 71);
AGCCAGCAGC (SEQ ID NO: 72); GCCAGCAGCA (SEQ ID NO: 73);
CCAGCAGCAG (SEQ ID NO: 74); CAGCAGCAGA (SEQ ID NO: 75);
AGCAGCAGAT (SEQ ID NO: 76); GCAGCAGATG (SEQ ID NO: 77);
CAGCAGATGG (SEQ ID NO: 78); AGCAGATGGG (SEQ ID NO: 79);
GCAGATGGGG (SEQ ID NO: 80); CAGATGGGGT (SEQ ID NO: 81);
AGATGGGGTG (SEQ ID NO: 82); GATGGGGTGG (SEQ ID NO: 83);
ATGGGGTGGG (SEQ ID NO: 84); TGGGGTGGGA (SEQ ID NO: 85);
GGGGTGGGAG (SEQ ID NO: 86); GGGTGGGAGC (SEQ ID NO: 87);
GGTGGGAGCA (SEQ ID NO: 88); GTGGGAGCAG (SEQ ID NO: 89);
TGGGAGCAGT (SEQ ID NO: 90); GGGAGCAGTA (SEQ ID NO: 91);
GGAGCAGTAT (SEQ ID NO: 92); GAGCAGTATC (SEQ ID NO: 93);
AGCAGTATCT (SEQ ID NO: 94); GCAGTATCTC (SEQ ID NO: 95);
CAGTATCTCG (SEQ ID NO: 96); AGTATCTCGA (SEQ ID NO: 97);
GTATCTCGAG (SEQ ID NO: 98); TATCTCGAGA (SEQ ID NO: 99);

ATCTCGAGAC(SEQ ID NO: 100); TCTCGAGACC(SEQ ID NO: 101);
CTCGAGACCT(SEQ ID NO: 102); TCGAGACCTA(SEQ ID NO: 103);
CGAGACCTAG(SEQ ID NO: 104); GAGACCTAGA(SEQ ID NO: 105);
AGACCTAGAA(SEQ ID NO: 106); GACCTAGAAA(SEQ ID NO: 107);
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CTAGAAAAAC(SEQ ID NO: 110); TAGAAAAACA(SEQ ID NO: 111);
AGAAAAACAT(SEQ ID NO: 112); GAAAAACATG(SEQ ID NO: 113);
AAAAACATGG(SEQ ID NO: 114); AAAACATGGA(SEQ ID NO: 115);
AAACATGGAG(SEQ ID NO: 116); AACATGGAGC(SEQ ID NO: 117);
ACATGGAGCA(SEQ ID NO: 118); CATGGAGCAA(SEQ ID NO: 119);
ATGGAGCAAT(SEQ ID NO: 120); TGGAGCAATC(SEQ ID NO: 121);
GGAGCAATCA(SEQ ID NO: 122); GAGCAATCAC(SEQ ID NO: 123);
AGCAATCACA(SEQ ID NO: 124); GCAATCACAA(SEQ ID NO: 125);
CAATCACAAG(SEQ ID NO: 126); AATCACAAGT(SEQ ID NO: 127);
ATCACAAGTA(SEQ ID NO: 128); TCACAAGTAG(SEQ ID NO: 129);
CACAAGTAGC(SEQ ID NO: 130); ACAAGTAGCA(SEQ ID NO: 131);
CAAGTAGCAA(SEQ ID NO: 132); AAGTAGCAAT(SEQ ID NO: 133);
AGTAGCAATA(SEQ ID NO: 134); GTAGCAATAC(SEQ ID NO: 135);
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GCAATACAGC(SEQ ID NO: 138); CAATACAGCA(SEQ ID NO: 139);
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CAGCAGCTAA(SEQ ID NO: 144); AGCAGCTAAC(SEQ ID NO: 145);
GCAGCTAACA(SEQ ID NO: 146); CAGCTAACAA(SEQ ID NO: 147);
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CTAACAATGC(SEQ ID NO: 150); TAACAATGCT(SEQ ID NO: 151);
AACAATGCTG(SEQ ID NO: 152); ACAATGCTGC(SEQ ID NO: 153);
CAATGCTGCT(SEQ ID NO: 154); AATGCTGCTT(SEQ ID NO: 155);
ATGCTGCTTG(SEQ ID NO: 156); TGCTGCTTGT(SEQ ID NO: 157);
GCTGCTTGTG(SEQ ID NO: 158); CTGCTTGTGC(SEQ ID NO: 159);
TGCTTGTGCC(SEQ ID NO: 160); GCTTGTGCCT(SEQ ID NO: 161);
CTTGTGCCTG(SEQ ID NO: 162); TTGTGCCTGG(SEQ ID NO: 163);
TGTGCCTGGC(SEQ ID NO: 164); GTGCCTGGCT(SEQ ID NO: 165);
TGCCTGGCTA(SEQ ID NO: 166); GCCTGGCTAG(SEQ ID NO: 167);
CCTGGCTAGA(SEQ ID NO: 168); CTGGCTAGAA(SEQ ID NO: 169);
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AGAACACCAG (SEQ ID NO: 480); GAACACCAGC (SEQ ID NO: 481);
AACACCAGCT (SEQ ID NO: 482); ACACCAGCTT (SEQ ID NO: 483);
CACCAGCTTG (SEQ ID NO: 484); ACCAGCTTGT (SEQ ID NO: 485);
CCAGCTTGTT (SEQ ID NO: 486); CAGCTTGTTA (SEQ ID NO: 487);
AGCTTGTTAC (SEQ ID NO: 488); GCTTGTTACA (SEQ ID NO: 489);
CTTGTTACAC (SEQ ID NO: 490); TTGTTACACC (SEQ ID NO: 491);
TGTTACACCC (SEQ ID NO: 492); GTTACACCCT (SEQ ID NO: 493);
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GAGGTTTGAC (SEQ ID NO: 550); AGGTTTGACA (SEQ ID NO: 551);
GGTTTGACAG (SEQ ID NO: 552); GTTTGACAGC (SEQ ID NO: 553);
TTTGACAGCC (SEQ ID NO: 554); TTGACAGCCG (SEQ ID NO: 555);

TGACAGCCGC(SEQ ID NO: 556); GACAGCCGCC(SEQ ID NO: 557);
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AGCCGCCTAG(SEQ ID NO: 560); GCCGCCTAGC(SEQ ID NO: 561);
CCGCCTAGCA(SEQ ID NO: 562); CGCCTAGCAT(SEQ ID NO: 563);
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AGCATTTCAT(SEQ ID NO: 568); GCATTTCATC(SEQ ID NO: 569);
CATTTCATCA(SEQ ID NO: 570); ATTTCATCAC(SEQ ID NO: 571);
TTTCATCACG(SEQ ID NO: 572); TTCATCACGT(SEQ ID NO: 573);
TCATCACGTG(SEQ ID NO: 574); CATCACGTGG(SEQ ID NO: 575);
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CCGAGAGCTG(SEQ ID NO: 586); CGAGAGCTGC(SEQ ID NO: 587);
GAGAGCTGCA(SEQ ID NO: 588); AGAGCTGCAT(SEQ ID NO: 589);
GAGCTGCATC(SEQ ID NO: 590); AGCTGCATCC(SEQ ID NO: 591);
GCTGCATCCG(SEQ ID NO: 592); CTGCATCCGG(SEQ ID NO: 593);
TGCATCCGGA(SEQ ID NO: 594); GCATCCGGAG(SEQ ID NO: 595);
CATCCGGAGT(SEQ ID NO: 596); ATCCGGAGTA(SEQ ID NO: 597);
TCCGGAGTAC(SEQ ID NO: 598); CCGGAGTACT(SEQ ID NO: 599);
CGGAGTACTT(SEQ ID NO: 600); GGAGTACTTC(SEQ ID NO: 601);
GAGTACTTCA(SEQ ID NO: 602); AGTACTTCAA(SEQ ID NO: 603);
GTACTTCAAG(SEQ ID NO: 604); TACTTCAAGA(SEQ ID NO: 605);
ACTTCAAGAA(SEQ ID NO: 606); CTTCAAGAAC(SEQ ID NO: 607);
TTCAAGAACT(SEQ ID NO: 608); TCAAGAACTG(SEQ ID NO: 609);
CAAGAACTGC(SEQ ID NO: 610); AAGAACTGCT(SEQ ID NO: 611);
AGAACTGCTG(SEQ ID NO: 612); GAACTGCTGA(SEQ ID NO: 613).

In a preferred embodiment the present invention contemplates a viral isolate which:

(i) is reactive to antibodies to a glycoprotein from HIV-1 such as at least one of gp41-45, gp120 and/or gp160;

(ii) carries a deletion of at least ten nucleotides in a region corresponding to the *nef* gene in HIV-1_{NL43}; and

wherein said deletion encompasses one or more decanucleotides from the *nef* gene of HIV-1_{NL43} or corresponding sequences from another pathogenic strain f HIV-1

defined in (or substantially analogous to) SEQ ID NOs:803 to 841:

ACCAGCTTGT	[SEQ ID NO:803]	CCAGCTTGTT [SEQ ID NO:804]
CAGCTTGTTA	[SEQ ID NO:805]	AGCTTGTTAC [SEQ ID NO:806]
GCTTGTTACA	[SEQ ID NO:807]	CTTGTTACAC [SEQ ID NO:808]
TTGTTACACC	[SEQ ID NO:809]	TGTTACACCC [SEQ ID NO:810]
GTTACACCCT	[SEQ ID NO:811]	TTACACCCTG [SEQ ID NO:812]
TACACCCTGT	[SEQ ID NO:813]	ACACCCTGTG [SEQ ID NO:814]
CACCCTGTGA	[SEQ ID NO:815]	ACCCTGTGAG [SEQ ID NO:816]
CCCTGTGAGC	[SEQ ID NO:817]	CCTGTGAGCC [SEQ ID NO:818]
CTGTGAGCCT	[SEQ ID NO:819]	TGTGAGCCTG [SEQ ID NO:820]
GTGAGCCTGC	[SEQ ID NO:821]	TGAGCCTGCA [SEQ ID NO:822]
GAGCCTGCAT	[SEQ ID NO:823]	AGCCTGCATG [SEQ ID NO:824]
GCCTGCATGG	[SEQ ID NO:825]	CCTGCATGGA [SEQ ID NO:826]
CTGCATGGAA	[SEQ ID NO:827]	TGCATGGAAT [SEQ ID NO:828]
GCATGGAATG	[SEQ ID NO:829]	CATGGAATGG [SEQ ID NO:830]
ATGGAATGGA	[SEQ ID NO:831]	TGGAATGGAT [SEQ ID NO:832]
GGAATGGATG	[SEQ ID NO:833]	GAATGGATGA [SEQ ID NO:834]
AATGGATGAC	[SEQ ID NO:835]	ATGGATGACC [SEQ ID NO:836]
TGGATGACCC	[SEQ ID NO:837]	GGATGACCCT [SEQ ID NO:838]
GATGACCCTG	[SEQ ID NO:839]	ATGACCCTGA [SEQ ID NO:840]
TGACCCTGAG	[SEQ ID NO:841]	

Generally, the subject HIV-1 isolate is non-pathogenic as hereinbefore defined. Additionally, reference herein to "a deletion" includes reference to a contiguous or non-contiguous series of two or more deletions.

The non-pathogenic isolate may carry a single decanucleotide deletion or may carry more than one decanucleotide deletion. Where it carries multiple deletions these may all correspond to a contiguous sequence or be from different parts of the *nef* gene. Furthermore, the terminal end portions of a deletion may lie within a decanucleotide as defined above. It is emphasised that the present invention extends to analogous sequences from other pathogenic strains of HIV-1 which might carry *nef* genes with a slightly altered sequence relative to HIV-1_{NL43}.

In a most preferred embodiment of the present invention, there is provided a non-pathogenic strain of HIV-1 carrying a nucleotide sequence in its genome as set forth in SEQ ID No. 614:

GAAGAGATTTGGGAGAACATGACCTGGATGCAGTGGGAAAAAGAAATTCACAATCACAC
AAAATACATATACTCCTTACTTGAAAAATCGCAGAACCAACAAGAAAAGAATGAACAAG
AACTATTGGAATTGGATCAATGGGCAAGTTTGTGGAATTGGTTTGACATAACAAAATGG
CTGTGGTATATAAAAATATTCATAATGGTAGTAGGAGGCTTGATAGGTTTAAGAATAGT
TTTTGCTGTACTTTCTATAGTGAATAGAGTTAGGCAGGGATACTCACCATTGTCGTTTC
AGACCCTCCTCCCAACCCCGAGGGGACCCGACAGGCCCGAAGGAATCGAAGAAGAAGGT
GGAGAGAGAGACAGAGACAGATCCACTCGATTAGTACACGGATTCTTAGCACTTTTCTG
GGACGACCTGAGGAGCCTGTGCCTCTTCCTCTACCACCACTTGAGAGACTTACTCTTGA
TTGTAACAAGGATTGTGGAACTTCTGGGACGCAGGGGATGGGAAGCCCTCAAATATTGG
TGGAACCTCCTAAAGTATTGGAGCCAGGAACTGCAGAAGAGTGCTGTTATCTTGCTCAA
TGCCACCGCCATAGCAGTAGCTGAGGGGACAGATAGAGTTTTAGAAGTATTACAAAGAG
CTTATAGAGCTATCCTCCACATACCTAGAAGAATAAGACAGGGCCTCGAAATGGCTTTG
CTATAAAATGGGTGGCAAGTGAGCAAAAAGTAGTGTAGTCAGATAGCATGCATCATAAG
GGGTGGGGGCCAACAACTAACAATGCTGATCGTGCCTGGCTAGAAGCACAAGAGAAGGA
AGAAGCGGGTTTTCCAGTCAAACCTCAGGTAGCTGTAGATCTTAGCCACTTTTTAAAAG
AAAAGGGGGGACTGGAAGGGCTAATTCACTCCCAAAGAAGACAAGATACACAGTGCTGC
AAACTATTACCAGTGGAGTCAGCGAAGATAGAAGAGGCCAATGGAGGAGAAAACCACAG
ATTGTTCTGTTGGGGACTTTCCATCCGTTGGGGACTTTCCAAGGCGGCGTGGCCTGGGT
GACTAGTTCCGGTGGGGACTTTCCAAGAAGJCGCGGCCTGGGCGGGACTGGGGAGTGGC
GAGCCCTCAGATGCTGCATATAAGCAGCTGCTTTCTGCTGTTACTGGGTCTCTCGGGTT
AGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTC
AATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGT
ATCTAGA;

and/or SEQ ID NO: 615:

GAAACAATTTGGGATAACATGACCTGGATGCAGTGGGAAAGAGAAATTGACAATTACAC
AAACATAATATACACCTTAATTGAAGAATCGCAGAACCAACAAGAAAAAAATGAACTAG
AATTATTGGAATTGGATAAATGGGCAAATTTGTGGAATTGGTTTAGTATATCAAACTGG
CTATGGTATATAAAATTATTCATAATGGTAGTAGGAGGCTTGGTAGGTTTAAGAATAGT
TTTTACTGTACTTTCTATAGTGAATAGAGTTAGGCAGGGATACTCACCATTGTCGTTTC
AGACCCACCTCCCAACCCCGAAGGGACCCGACAGGCCAGAAGGAATCGAAGAAGAAGGT
GGAGAGAGAGACAGAGGCAGCTCCACTCGATTAGTGCACGGATTCTTAGCACTTTTCTG
GGACGACCTGAGGAGTCTGTGCCTCTTCAGCTACCACCACTTGAGAGACTTACTCTTGA

TTGTAACGAGGATTGTGGAACTTCTGGGACGCAGGGGATGGGAAGCCCTCAAATACTGG
TGGAATCTCCTGCAGTATTGGAGGCAGGAACTACAGAAGAGTGCTGTTAGCTTGTTCAA
TGGCACGGCCATAGCAGTAGCTGAGGGGACAGATAGAGTTATAGAAGCTTTACGAAGGG
CTTATAGAGCTATTCTCCACATACCTAGAAGAATAAGACAGGGCTTAGAAAGGGCTTTG
CTATAAAATGGGTGGCAAGTGGTCAGAAAGTAGTGTGGTTAGAAGGCATGTACCTTTAA
GACAAGGCAGCTATAGATCTTAGCCGCTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCT
AATTCACTCACAGAGAAGATCAGTTGAACCAGAAGAAGATAGAAGAGGCCATGAAGAAG
AAAACAACAGATTGTTCCGTTTGTTCCGTTGGGGACTTTCCAGGAGACGTGGCCTGAGT
GATAAGCCGCTGGGGACTTTCCGAAGAGGCGTGACGGGACTTTCCAAGGCGACGTGGCC
TGGGCGGGACTGGGGAGTGGCGAGCCCTCAGATGCTGCATATAAGCAGCTGCTTTCTGC
CTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAG
GGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCC
CGTCTGTTGTGTGACTCTGGTATCTAGA.

The present invention, however, extends to HIV-1 isolates which are non-pathogenic; carry genomes capable of hybridising under low stringency conditions to SEQ ID NO: 614 or SEQ ID NO: 615; and which do not direct the synthesis of a full length *nef* gene product.

In a further embodiment the present invention contemplates a viral isolate which:

(i) is reactive to antibodies to a glycoprotein from HIV-1 such as at least one f gp41-45, gp120 and/or gp160;

(ii) carries a genome or a part or fragment thereof capable of hybridising under medium stringency conditions to a nucleotide sequence as set forth in SEQ ID NO: 1 or a complementary form thereof or an analogous sequence from another pathogenic strain of HIV-1;

(iii) carries a deletion of at least ten nucleotides in a region corresponding to the LTR region in HIV-1_{NL43}; and

wherein said deletion encompasses one or more of the following decanucleotides from the LTR region of HIV-1_{NL43} or corresponding sequences from another pathogenic strain of HIV-1:

GCTTTTTGCC (SEQ ID NO: 652); CTTTTTGCCT (SEQ ID NO: 653);
TTTTTGCCTG (SEQ ID NO: 654); TTTTGCCTGT (SEQ ID NO: 655);
TTTGCCTGTA (SEQ ID NO: 656); TTGCCTGTAC (SEQ ID NO: 657);

TGCCTGTACT (SEQ ID NO: 658); GCCTGTACTG (SEQ ID NO: 659);
CCTGTACTGG (SEQ ID NO: 660); CTGTACTGGG (SEQ ID NO: 661);
TGTACTGGGT (SEQ ID NO: 662); GTACTGGGTC (SEQ ID NO: 663);
TACTGGGTCT (SEQ ID NO: 664); ACTGGGTCTC (SEQ ID NO: 665);
CTGGGTCTCT (SEQ ID NO: 666); TGGGTCTCTC (SEQ ID NO: 667);
GGGTCTCTCT (SEQ ID NO: 668); GGTCTCTCTG (SEQ ID NO: 669);
GTCTCTCTGG (SEQ ID NO: 670); TCTCTCTGGT (SEQ ID NO: 671);
CTCTCTGGTT (SEQ ID NO: 672); TCTCTGGTTA (SEQ ID NO: 673);
CTCTGGTTAG (SEQ ID NO: 674); TCTCTGGTTA (SEQ ID NO: 675);
CTGGTTAGAC (SEQ ID NO: 676); TGGTTAGACC (SEQ ID NO: 677);
GGTTAGACCA (SEQ ID NO: 678); GTTAGACCAG (SEQ ID NO: 679);
TTAGACCAGA (SEQ ID NO: 680); TAGACCAGAT (SEQ ID NO: 681);
AGACCAGATC (SEQ ID NO: 682); GACCAGATCT (SEQ ID NO: 683);
ACCAGATCTG (SEQ ID NO: 684); CCAGATCTGA (SEQ ID NO: 685);
CAGATCTGAG (SEQ ID NO: 686); AGATCTGAGC (SEQ ID NO: 687);
GATCTGAGCC (SEQ ID NO: 688); ATCTGAGCCT (SEQ ID NO: 689);
TCTGAGCCTG (SEQ ID NO: 690); CTGAGCCTGG (SEQ ID NO: 691);
TGAGCCTGGG (SEQ ID NO: 692); GAGCCTGGGA (SEQ ID NO: 693);
AGCCTGGGAG (SEQ ID NO: 694); GCCTGGGAGC (SEQ ID NO: 695);
CCTGGGAGCT (SEQ ID NO: 696); CTGGGAGCTC (SEQ ID NO: 697);
TGGGAGCTCT (SEQ ID NO: 698); GGGAGCTCTC (SEQ ID NO: 699);
GGAGCTCTCT (SEQ ID NO: 700); GAGCTCTCTG (SEQ ID NO: 701);
AGCTCTCTGG (SEQ ID NO: 702); GCTCTCTGGC (SEQ ID NO: 703);
CTCTCTGGCT (SEQ ID NO: 704); TCTCTGGCTA (SEQ ID NO: 705);
CTCTGGCTAA (SEQ ID NO: 706); TCTGGCTAAC (SEQ ID NO: 707);
CTGGCTAACT (SEQ ID NO: 708); TGGCTAACTA (SEQ ID NO: 709);
GGCTAACTAG (SEQ ID NO: 710); GCTAACTAGG (SEQ ID NO: 711);
CTAACTAGGG (SEQ ID NO: 712); TAACTAGGGA (SEQ ID NO: 713);
AACTAGGGAA (SEQ ID NO: 714); ACTAGGGAAC (SEQ ID NO: 715);
CTAGGGAACC (SEQ ID NO: 716); TAGGGAACCC (SEQ ID NO: 717);
AGGGAACCCA (SEQ ID NO: 718); GGGAACCCAC (SEQ ID NO: 719);
GGAACCCACT (SEQ ID NO: 720); GAACCCACTG (SEQ ID NO: 721);
AACCCACTGC (SEQ ID NO: 722); ACCCACTGCT (SEQ ID NO: 723);
CCCACTGCTT (SEQ ID NO: 724); CCACTGCTTA (SEQ ID NO: 725);
CACTGCTTAA (SEQ ID NO: 726); ACTGCTTAAG (SEQ ID NO: 727);
CTGCTTAAGC (SEQ ID NO: 728); TGCTTAAGCC (SEQ ID NO: 729);
GCTTAAGCCT (SEQ ID NO: 730); CTTAAGCCTC (SEQ ID NO: 731);
TTAAGCCTCA (SEQ ID NO: 732); TAAGCCTCAA (SEQ ID NO: 733);

AAGCCTCAAT (SEQ ID NO: 734); AGCCTCAATA (SEQ ID NO: 735);
GCCTCAATAA (SEQ ID NO: 736); CCTCAATAAA (SEQ ID NO: 737);
CTCAATAAAG (SEQ ID NO: 738); TCAATAAAGC (SEQ ID NO: 739);
CAATAAAGCT (SEQ ID NO: 740); AATAAAGCTT (SEQ ID NO: 741);
ATAAAGCTTG (SEQ ID NO: 742); TAAAGCTTGC (SEQ ID NO: 743);
AAAGCTTGCC (SEQ ID NO: 744); AAGCTTGCCT (SEQ ID NO: 745);
AGCTTGCCTT (SEQ ID NO: 746); GCTTGCCTTG (SEQ ID NO: 747);
CTTGCCTTGA (SEQ ID NO: 748); TTGCCTTGAG (SEQ ID NO: 749);
TGCCTTGAGT (SEQ ID NO: 750); GCCTTGAGTG (SEQ ID NO: 751);
CCTTGAGTGC (SEQ ID NO: 752); CTTGAGTGCT (SEQ ID NO: 753);
TTGAGTGCTT (SEQ ID NO: 754); TGAGTGCTTC (SEQ ID NO: 755);
GAGTGCTTCA (SEQ ID NO: 756); AGTGCTTCAA (SEQ ID NO: 757);
GTGCTTCAAG (SEQ ID NO: 758); TGCTTCAAGT (SEQ ID NO: 759);
GCTTCAAGTA (SEQ ID NO: 760); CTTCAAGTAG (SEQ ID NO: 761);
TTCAAGTAGT (SEQ ID NO: 762); TCAAGTAGTG (SEQ ID NO: 763);
CAAGTAGTGT (SEQ ID NO: 764); AAGTAGTGTG (SEQ ID NO: 765);
AGTAGTGTGT (SEQ ID NO: 766); GTAGTGTGTG (SEQ ID NO: 767);
TAGTGTGTGC (SEQ ID NO: 768); AGTGTGTGCC (SEQ ID NO: 769);
GTGTGTGCCC (SEQ ID NO: 770); TGTGTGCCCG (SEQ ID NO: 771);
GTGTGCCCGT (SEQ ID NO: 772); TGTGCCCGTC (SEQ ID NO: 773);
GTGCCCGTCT (SEQ ID NO: 774); TGCCCGTCTG (SEQ ID NO: 775);
GCCCGTCTGT (SEQ ID NO: 776); CCCGTCTGTT (SEQ ID NO: 777);
CCGTCTGTTG (SEQ ID NO: 778); CGTCTGTTGT (SEQ ID NO: 779);
GTCTGTTGTG (SEQ ID NO: 780); TCTGTTGTGT (SEQ ID NO: 781);
CTGTTGTGTG (SEQ ID NO: 782); TGTTGTGTGA (SEQ ID NO: 783);
GTTGTGTGAC (SEQ ID NO: 784); TTGTGTGACT (SEQ ID NO: 785);
TGTGTGACTC (SEQ ID NO: 786); GTGTGACTCT (SEQ ID NO: 787);
TGTGTGACTC (SEQ ID NO: 788); GTGTGACTCT (SEQ ID NO: 789);
TGTGACTCTG (SEQ ID NO: 790); GTGACTCTGG (SEQ ID NO: 791);
TGACTCTGGT (SEQ ID NO: 792); GACTCTGGTA (SEQ ID NO: 793);
ACTCTGGTAA (SEQ ID NO: 794); CTCTGGTAAC (SEQ ID NO: 795);
TCTGGTAACT (SEQ ID NO: 796); CTGGTAACTA (SEQ ID NO: 797);
TGGTAACTAG (SEQ ID NO: 798); GGTAACTAGA (SEQ ID NO: 799).

The non-pathogenic isolate may carry a single decanucleotide deletion in the LTR region or may carry multiple deletions in the same region or in the LTR region and another region such as the *nef* gene. In particular, the mutation may be in the LTR/*nef* overlap

region. Where it carries multiple deletions, these may correspond to a contigu us sequence or be from different parts of the LTR region and/or *nef* gene. Furthermore, the terminal end portions of a deletion may lie within a decanucleotide as defined above.

Yet another aspect of the present invention provides an infectious molecular clone comprising genetic sequences derived from the non-pathogenic HIV-I isolates as hereinbefore described and includes genetic sequences encoding major structural proteins such as *gag, env* and *pol*. Infectious molecular clones are particularly useful as genetic compositions capable of "infecting" host cells without need of viral coat. The infectious molecular clones of the present invention may also be derived from pathogenic HIV-1 strains rendered non-pathogenic as hereindescribed.

According to this latter embodiment, there is contemplated a method of attenuating a pathogenic strain of HIV-1, said method comprising inducing a mutation in the *nef* gene and/or an LTR region to generate a non-pathogenic HIV-1 strain as hereinbefore described. Preferred mutations are deletions of at least ten nucleotides such as one or more of the decanucleotides as hereinbefore described. Particularly preferred mutations result in modified Nef protein carrying a deletion substantially corresponding to amino acids 162 to 177 of Nef from wild-type HIV-1. The mutations may also constitute substitutions and/or insertions of heterologous nucleic acid molecules in the *nef* and/or LTR regions such as the incorporation of a sense (i.e. co-suppression) or antisense molecule.

In still yet another embodiment of the present invention, there is provided an isolated, non-pathogenic strain of HIV-1 comprising a deletion in its genome of at least 10 nucleotide within the region of nucleotide 8787 and 9709 and more particularly within the region 8800 and 9700 and even more preferably within the region 8800 and 9410, using the nucleotide numbering of HIV-1_{NL43}.

In one embodiment, the deletion of at least 10 nucleotides is from within a region selected from the list consisting of (using the nucleotide numbers of HIV-1_{NL43}):

nucleotide (i) 8830-8862;
(ii) 9009-9035;
(iii) 9019-9029; and
(iv) 9033-9049.

In another embodiment, the deletion of at least 10 nucleotides is from within a region selected from the list consisting of (using the nucleotide numbers of HIV-1_{NL43}):

nucleotide (v) 9281-9371;
(vi) 9281-9362;
(vii) 9105-9224; and
(viii) 9271-9370.

In yet another embodiment, the deletion of at least 10 nucleotides is from within a region selected from the list consisting of (using the nucleotide numbers of HIV-1_{NL43}):

nucleotide (ix) 8882-8928;
(x) 8850-9006;
(xi) 8792-9041; and
(xii) 9112-9204.

In still yet another embodiment, the deletion of at least 10 nucleotides is from within a region selected from the list consisting of (using the nucleotide numbers of HIV-1_{NL43}):

nucleotide (xiii) 9105-9224;
(xiv) 9389-9395; and
(xv) 9281-9366.

The above embodiments including any combinations thereof or functional equivalents thereof. Most preferred variants of HIV-1 are defined in Table 3.

Reference herein to the non-pathogenic HIV-1 strains of the present invention includes reference to components, parts, fragments and derivatives thereof including both genetic and non-genetic material. Furthermore, the non-pathogenic HIV-1 strains may be in isolated form or resident in peripheral blood mononuclear cells (PBMCs) or other like cells where the genome of the HIV-1 strains is integrated as DNA from said HIV-1 strains such as proviral DNA. In addition, the present invention extends to recombinant virus such as from (or resident in) prokaryotes or eukaryotes as well as in the form of infectious molecular clones.

Accordingly, the present invention provides for the non-pathogenic HIV-1 isolate, genomic material therefrom, complementary proviral DNA, molecular infectious clones, recombinant viral particles or genetic sequences therefrom or cells expressing same or blood cells carrying proviral DNA or to any mutants, derivatives, components, fragments, parts, homologues or analogues of the foregoing.

Another aspect of the present invention contemplates a synthetic peptide comprising a sequence of amino acids as defined in SEQ ID NO:699 or a part or a fragment thereof. The synthetic peptide generally comprises at least four, preferably at least five, more preferably at least six and even more preferably at least seven or more of the amino acids as defined in SEQ ID NO:699. Furthermore, the synthetic peptides may comprise chemically modified amino acids or structurally, functionally and/or stereochemically equivalent substitute amino acids. Such synthetic peptides are useful in diagnostic protocols or in therapeutic regimums, such as in generating antibodies to that particular region of the Nef protein. By administering the synthetic peptides of this aspect of the present invention, higher titres of antibodies to a particular region of Nef may be obtained compared to what would be stimulated if a larger portion of Nef is used.

The non-pathogenic HIV-1 strains of the present invention are particularly useful in the development of therapeutic compositions, therapeutic molecules and/or diagnostic reagents. With regards to the former, the non-pathogenic HIV-1 strain may be considered as a live attenuated vaccine where individuals carrying DNA derived from

said non-pathogenic HIV-1 strain such as proviral DNA in target cells are protected from infection by a corresponding pathogenic strain. The term "vaccine" is used in its broadest sense as a therapeutic composition or molecule which prevents or reduces HIV-1 infection or risk of infection or which ameliorates the symptoms of infection. It may involve the stimulation of an immune response or may involve blocking HIV-1 cells receptors and/or the use of genetic compositions, for example, to introduce ribozymes or antisense molecules to HIV-1 directed genetic sequences or to prepare infectious molecular clones. For convenience, all such compositions are referred hereinafter to "therapeutic compositions".

Accordingly, the present invention contemplates a method for inhibiting or reducing the risk of infection by a pathogenic strain of HIV-1, said method comprising administering to a subject a non-pathogenic HIV-1 as hereinbefore defined in an amount effective to infect target cells and to generate target cells carrying proviral DNA from said non-pathogenic HIV-1. More particularly, the present invention contemplates a method for inhibiting or reducing productive infection of an individual by a pathogenic strain of HIV-1, said method comprising administering to a subject a non-pathogenic isolate of HIV-1 in an amount effective to infect target cells and to generate target cells carrying proviral DNA from said non-pathogenic HIV-1. By "productive infection" as used in the specification and claims herein is meant the infection of a cell or cells by a pathogenic strain of HIV-1 which leads ultimately to the symptomology of AIDS or AIDS related diseases. A cell infected productively produces pathogenic virions. By definition, infection of an individual by a non-pathogenic strain of HIV-1 would not lead to productive infection. Non-pathogenic HIV-1 strains generally replicate to a sufficient extent to protect cells against challenge by virulent or pathogenic strains. The methods of the present invention are applicable prophylactically (i.e. to prevent *de novo* infection) or therapeutically (i.e. to reduce or slow disease progression).

The present invention further provides a method for vaccinating an individual against the development of AIDS or AIDS-related diseases, said method comprising administering to said individual a non-pathogenic isolate of HIV-1 in an amount effective to infect target cells and to generate target cells carrying proviral DNA from

said non-pathogenic HIV-1. The term "vaccinating" should not be taken as limiting the invention to the prevention of HIV-1 infection by solely immunological means. The term "vaccinating" includes any means of preventing productive infection of an individual by pathogenic HIV-1. Particularly, preferred non-pathogenic strains of HIV-1 according to these aspects of the present invention are generally defined as encoding a modified protein such as a modified Nef protein and in particular a modified Nef protein which is substantially non-interactive to antibodies to amino acids 162 to 177 of wild-type HIV-1 Nef.

As an alternative to the above methods, a therapeutic composition as hereinbefore defined is administered. The non-pathogenic isolate may be administered *inter alia* as an isolated viral preparation or *via* infected blood cells. Another aspect of the invention provides a therapeutic composition for inhibiting or reducing the risk of infection by a pathogenic strain of HIV-1 said therapeutic composition comprising a non-pathogenic strain of HIV-1 or genetic sequences derived therefrom as hereinbefore described and optionally one or more pharmaceutically acceptable carriers and/or diluents. In a further embodiment, the therapeutic compositions comprise the synthetic peptides comprising an amino acid sequence as set forth in SEQ ID NO:699 or a part, fragment or homologue thereof. Such a vaccine would generate high titre antibodies to a specific region of Nef protein.

The therapeutic composition of the present invention is generally suitable for intravenous, intraperitoneal, intramuscular, intramucosal (e.g. nasal spray, respiratory spray) or other forms of parenteral administration. The therapeutic composition might also be administered *via* an implant or rectally or orally. In addition to the mutations contemplated above, the non-pathogenic HIV-1 strain may also contain one or more other mutations to further reduce the risk of reversion to virulence and/or to insert a genetic sequence capable of providing directly or indirectly an identifiable signal, having further anti-HIV-1 properties and/or immunostimulatory or cell regulatory properties.

For example, the non-pathogenic HIV-1 isolate in the therapeutic composition may comprise additional genetic material capable of directing expression of antisense nucleotide sequences to inhibit expression of one or more proteins encoded by a pathogenic strain of HIV-1. Alternatively, sense co-suppression may be employed. Preferred sense or antisense molecules would reduce expression of the *nef* gene or affect normal functioning of the LTR region. In a particularly preferred embodiment, the nucleotide sequence encoding amino acids 162 to 177 is targetted by sense or antisense molecules.

According to this embodiment, the non-pathogenic HIV-1 strain may be considered as a targeting agent to introduce genetic constructs capable of reducing expression of one or more HIV-1 proteins or polypeptides. In this embodiment there is provided a viral isolate which:

(i) is genetically or immunologically related to a pathogenic strain of HIV-1;

(ii) is substantially non-pathogenic in human subjects;

(iii) comprises a first nucleotide sequence constituting its genome which is capable of hybridising under medium stringency conditions to SEQ ID NO: 1 or a complementary form thereof; and

(iv) comprises a second nucleotide sequence within said first nucleotide sequence and which second nucleotide sequence directs expression of a mRNA molecule capable of inhibiting, reducing or otherwise down-regulating translation of a protein or polypeptide encoded by a pathogenic strain of HIV-1 or inhibit, reduce or otherwise down regulate operation of a non-protein encoding a region of a pathogenic strain of HIV-1.

Preferred proteins or polypeptides targeted for reduced expression are those encoded by one or more of the following: *gag, pol, env, tat, rev, vpu, vpr, vif* and/or *nef* genes. The most preferred protein or polypeptide targeted for reduced expression is the product of the *nef* gene. Alternatively, the target nucleotide sequence does not encode a polypeptide or protein but is required for other functions such as integration or excision from the human genome or expression f genes on the viral genome. An example f

such a nucleotide sequence is the LTR region. Accordingly, the present invention extends to disruption to the function of such regions.

In a particularly preferred embodiment there is provided a viral isolate which:

(i) is genetically or immunologically related to a pathogenic strain of HIV-1;

(ii) is substantially non-pathogenic in human subjects; and

(iii) comprises a nucleotide sequence which directs expression of a mRNA molecule capable of inhibiting, reducing or otherwise down-regulating translation of Nef.

Preferably, the nucleotide sequence reduces levels of amino acids 162 to 177 in Nef. The above aspect relates to use of antisense technology. The present invention extends, however, to use of ribozymes and/or co-suppression to achieve the same results. In an alternative embodiment, or in addition to, the second (or optionally a third) nucleotide sequence encodes an antiviral agent (e.g. interferon) and/or an immune enhancing agent.

The identification of deletions *inter alia* in the *nef* gene and/or LTR region in asymptomatic subjects provides a unique opportunity to study the *in vivo* effects of attenuated HIV-1 strains carrying one or more mutations in selected genetic regions. In particular, the present invention provides a means for designing therapeutic compositions directed to inhibiting expression of a *nef* gene and/or LTR region in a pathogenic HIV-1 strain (such as contemplated above) as well as developing a therapeutic regimem aimed at inhibiting the activity of the *nef* gene product for the function of the LTR region. According to this latter embodiment, the present invention provides a therapeutic composition comprising a molecule capable of inhibiting the intracellular activity of the *nef* gene product and/or LTR region, said composition further comprising one or more pharmaceutically acceptable carriers and/or diluents. In one preferred embodiment, the inhibition effects amino acids 162 to 177 of Nef.

The molecule contemplated by the above aspect of the subject invention may be a protein, polypeptide, peptide, chemical compound, sugar moiety or derivative of the *nef* gene product. The molecule will need to be capable f entering an infected cell. Where the molecule is a protein, polypeptide or peptide, it may be encoded by the second

nucleotide sequence on the targeting vector as contemplated above. Alternatively, the molecule may be a nucleic acid molecule capable of targeting the *nef* gene or LTR region. Where the *nef* gene is targetted, the preferred region is the nucleotide sequence which encodes amino acids 162 to 177 of Nef.

The deletion mutants of the present invention may result in a modified *nef* gene product either having no readily discernable activity or having activity different to the naturally occurring *nef* protein. In any event, if a mutant *nef* gene product is produced, it will generally have a lower molecular weight than the naturally occurring *nef* protein and will have a different overall amino acid sequence. Importantly, it will be immunologically distinguishable from wild-type Nef in that it will be substantially non-interactive with antibodies to a particular region of Nef, such as amino acids 162 to 177 of wild-type Nef. This provides, therefore, for a means for diagnosing individuals with benign HIV-1 infection by, for example, assaying for a modified *nef* protein or screening for a modified *nef* gene sequence. Alternatively, benign HIV-1 infection may be detected by assaying for a modified LTR region such as an altered nucleotide sequence.

These aforementioned aspects of the present invention apply to screening deletion, truncation or other mutants of HIV-1-derived proteins where such mutations result in a strain of HIV-1 being substantially non-pathogenic. Although a variety of procedures are available to detect a modified HIV-1-derived protein, a particularly convenient approach is to screen HIV-1 infected individuals for the absence of antibodies to the deleted or truncated portion of a target protein.

According to one embodiment there is provided a method for determining the pathogenicity of a strain of HIV-1 after said HIV-1 strain infects cells of an individual said method comprising contacting a biological sample from said individual with a peptide corresponding to a deleted or truncated region of an HIV-1-derived protein and screening for the absence of antibody binding to said peptide, wherein the absence of antibody binding is indicative f a deletion or truncati n in that protein and further indicative f the non-pathogenicity f said strain of HIV-1.

Although this general methodology is applicable to any protein encoded by HIV-1 which is critical for pathogenicity in a host, it is particularly useful in screening deletions in the Nef protein. An exemplary deletion in the Nef protein is all or part of the sequence of amino acids 162 to 177 of Nef. Preferably, the assay would include a positive control such that where antibodies are present to Nef, such antibodies would bind to this positive control. When a Nef protein carries a deletion at or about amino acids 162 to 177, antibodies to this region would be absent in an individual infected by a non-pathogenic strain of HIV-1 and no binding would be detected to a peptide covering this region.

According to a particularly preferred embodiment, there is contemplated a method for determining the pathogenicity of an HIV-1 strain after said strain infects cells of an individual, said method comprising contacting a biological sample from said individual with an effective amount of a peptide having an amino acid sequence comprising or within amino acids 162 to 177 of wild-type HIV-1_{NL43} Nef, said contact being for a time and under conditions sufficient for an antibody if present in said biological sample to form a complex with said peptide and then detecting the presence of said complex wherein the absence of a complex is an individual seropositive for HIV-1 is indicative of that the individual being infected with a non-pathogenic strain of HIV-1. The method may also comprise contacting the biological sample with one or more peptides derived from other regions of Nef such as flanking regions to amino acids 162 to 177 where antibodies to such other regions may be expected even in non-pathogenic HIV-1 strains. This would provide a suitable positive control. A biological sample according to this embodiment would be any source of antibodies such as serum and whole blood. Preferably, the peptides are immobilized to a solid support as described below. This method of the present invention is also applicable for determining the risk of an individual seropositive for HIV-1 developing symptoms of AIDS. Again, substantial absence of antibodies to amino acids 162 to 177 of Nef in an HIV-1 seropositive individual would be indicative of a low risk of an individual developing AIDS.

Other methods of screening for the pathogenicity or otherwise of strains of HIV-1 readily become apparent as a result of the present invention. For example, antibodies may be first generated to modified Nef proteins from non-pathogenic strains of HIV-1 where such antibodies would not recognise wild-type Nef protein.

According to one embodiment, there is provided a method for determining the pathogenicity of an HIV-1 strain after said HIV-1 strain infects cells of an individual, said method comprising contacting a biological sample from said individual with an effective amount of an antibody specific to a *nef* protein from a non-pathogenic strain of HIV-1 (as hereinbefore defined) for a time and under conditions sufficient to form an antibody-modified *nef* protein complex and then detecting said complex. The presence of said complex is indicative of a modified *nef* gene product and of the non-pathogenicity of the strain of HIV-1. Alternatively, the isolated Nef could be tested against a panel of antibodies where certain antibodies are specific to a deleted region. The absence of antibody binding would be indicative of a deletion and, therefore, a modified Nef protein and in turn a putative non-pathogenic form of the virus. The biological sample is a sample likely to contain the modified *nef* gene product such as tissue extract or cell extract of an infected cell. However, where the modified *nef* gene product is capable of permeation or transport out of the cell, suitable biological fluid would include serum, whole blood, lymph and mucosal secretion amongst other fluids.

Many variations in the aforementioned assays are possible and are contemplated herein. For example, an assay could also be based on the inability for a *nef* specific antibody to bind to a modified *nef* protein. For the purposes of the present invention the term "contacting" including "mixing".

The presence of a modified *nef* molecule, such as a molecule carrying a deletion, or other suitable HIV-1 derived protein in biological fluid can be detected using a wide range of immunoassay techniques such as those described in US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. These include both single-site and two-site, or "sandwich", assays f the non-competitive types, as well as in the traditional competitive binding

assays and include ELISA and RIA techniques. Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, and by way of example only, in a typical forward assay, a modified *nef* product-specific antibody is immobilised onto a solid substrate to form a first complex and the sample to be tested for modified *nef* product brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-modified *nef* product secondary complex, a second modified *nef* protein antibody, labelled with a reporter molecule capable of producing a detectable signal, is then added and incubated, allowing time sufficient for the formation of a tertiary complex of antibody-modified *nef* product-antibody. Any unreacted material is washed away, and the presence of the tertiary complex is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal or may be quantitated by comparison with a control sample containing known amounts of hapten. Variations of the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody, or a reverse assay in which the labelled antibody and sample to be tested are first combined, incubated and then added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, and the possibility of minor variations will be readily apparent. The antibodies used above may be monoclonal or polyclonal. In a preferred embodiment, antibodies to Nef in an individual's biological fluid are screened for using peptides derived from Nef. The absence of antibodies to specific regions of Nef such as amino acids 162 to 177 would be indicative of a non-pathogenic strain of HIV-1. All such assays and variations of such assays are encompassed by the present invention.

The solid substrate is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs or microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist f cross-linking covalently binding or physically

adsorbing the molecule to the insoluble carrier.

By "reporter molecule", as used in the present specification, is meant a molecule which, by its chemical nature, produces an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes). In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist which are readily available to one skilled in the art. Commonly used enzymes include horseradish peroxidase, glucose oxidase, β-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production,upon hydrolysis by the corresponding enzyme, of a detectable colour change. It is also possible to employ fluorogenic substrates, which yield a fluorescent product.

Alternatively, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining complex is then exposed to the light of the appropriate wavelength, the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed. It will be readily apparent to the skilled technician how to vary the procedure to suit the required purpose. It will also be apparent that the foregoing can be used to label a modified *nef* product and to use same directly in the detection of, for example, circulatory antibodies specific to said modified *nef* product.

Alternatively, genetic assays may be conducted to screen for abberations in the *nef* gene and/or LTR region. Such a genetic assay may be by Southern or Northern blot analysis, PCR analysis or the like using oligonucleotides specific to a deleted region of a *nef* gene and/or LTR region.

According to this embodiment there is provided a method for determining the pathogenicity of an HIV-1 strain after said HIV-1 strain infects cells of an individual, said method comprising determining directly or indirectly the presence of a deletion mutation in the genome of said HIV-1 wherein the presence of a such a mutation is indicative of the presence of a non-pathogenic strain of HIV-1. The deletion mutation may result in the genome being unable to synthesize a polypeptide or protein from pathogenic strain of HIV-1 or may direct the synthesis of a truncated or deleted form of said polypeptide or protein. For example, a Nef protein with amino acids 162 to 177 deleted therefrom. The mutation may also lead to altered expression of a polypeptide detectable by, for example, decreased synthesis of a particular protein, such as the *nef* gene product. Alternatively, the deletion mutation affects the LTR region or a regulatory region of the HIV-1 genome. In either case, affected viruses may also be detected by, for example, observing low viral copy numbers such as low viral loads.

Preferably, said non-pathogenic HIV-1 carries a deletion in its genome of at least 10 nucleotides within the region from nucleotide 8787 to nucleotide 9709 using the nucleotide numbering of HIV-1_{NL43}.

Preferably, said non-pathogenic HIV-1 carries a deletion in its genome of at least 10 nucleotides from within a region selected from the list consisting of:

nucleotide	(i)	8830-8862;
	(ii)	9009-9035;
	(iii)	9019-9029; and
	(iv)	9033-9049.

Preferably, said non-pathogenic HIV-1 carries a deletion in its genome of at least 10 nucleotides from within a region selected from the list consisting of:

nucleotide	(v)	9281-9371;
	(vi)	9281-9362;
	(vii)	9105-9224; and
	(viii)	9271-9370.

Preferably, said non-pathogenic HIV-1 carries a deletion in its genome of at least 10 nucleotides from within a region selected from the list consisting of:

nucleotide	(ix)	8882-8928;
	(x)	8850-9006;
	(xi)	8792-9041; and
	(xii)	9112-9204.

Preferably, said non-pathogenic HIV-1 carries a deletion in its genome of at least 10 nucleotides from within a region selected from the list consisting of:

nucleotide	(xiii)	9105-9224;
	(xiv)	9389-9395; and
	(xv)	9281-9366.

The above nucleotide numbers are based on the nucleotide numbering in the NL43 genome.

Particularly preferred oligonucleotides are based on the deleted regions of the *nef* gene and/or LTR region such as but not limited to one or more oligonucleotides based on SEQ ID NO: 2 to SEQ ID NO: 613 and/or SEQ ID NO: 652 to SEQ ID NO: 799.

Most preferred deeltions include deletions of one or more of SEQ ID NO:803 to 841 which cover amino acids 162 to 177 of Nef. A particularly preferred genetic assay screens for this deletion.

The present invention further extends to kits for the diagnosis of infection by pathogenic strains of HIV-1 or for determining the pathogenicity of infecting virus. The kits would be in compartmental form each comprising one or more suitable reagents for conducting the assay.

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1

Source Material

For the purposes of the following examples, non-pathogenic HIV-1 strains were isolated from recipients of HIV-1 infected blood. The recipients are designated "C18", "C54", "C98", "C49", "C64" and "C124". The donor is identified herein as "D36". The place of isolation may be indicated after the abbreviation of "HIV". For example, St Vincents Hospital, Sydney (HIV_{StV}) or Macfarlane Burnet Centre of Medical Research, Melbourne (HIV_{MBC}).

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Exemplary viral isolates referred to herein as "C18" and "C98" were deposited at the PHLS Centre for Applied Microbiology and Research, European Collection of Animal Cell Cultures (ECACC), Division of Biologies, Porton Down, Salisbury, Wiltshire SP4 OJG. C18 was deposited on 17 October, 1994 under Provisional Accession Number V94101706 and C98 was deposited on 31 October, 1994 under Provisional Accession Number V941031169. Another isolate "C54" was deposited at ECACC on 10 March, 1995 under Provisional Accession Number V95031022.

Figure 11 is a summary of the deletion mutants of the present invention.

Viruses are isolatable by the following procedures:

1. Infected peripheral blood mononuclear cells (PBMCs) were co-cultured with HIV-1 seronegative donor PBMCs. A convenient source of seronegaitve donor PBMCs is a blood bank. The supernatants and cells are harvested every 7 days and fresh medium added with CD8 depleted PBMCs. CD8 depletion promotes the ability to isolate HIV-1. The culture and procedure is continued for up to

approximately 5 weeks;

2. The infected PBMCs are purified from whole blood and these cells are cultured alone for up to 5 weeks. PMBCs alone are used because the virus is more likely to be monocytotropic. Fresh medium is added weekly and supernatant fluid is harvested at this time;

3. Supernatant fluids are harvested every approximately 7 days, fresh medium and fresh HIV-1 seronegative CD8 depleted PBMCs are added at this time;

4. HIV-1 seronegative PBMCs are pretreated with with M-CSF for approximately 72 hours prior to the addition of infected PBMCs. M-CSF has been shown to enhance HIV-1 replication in monocytes (Gendelman *et al*, 1988); and

5. The supernatant fluid is harvested from the cultures of step 4 every approximately 7 days, fresh medium added together with HIV-1 seronegative stimulated CD8 depleted PBMCs. The virus is isolated from the infected PBMCs.

A particularly preferred method of isolation is as follows:
HIV negative donor PBMC were stimulated by culture in RPMI 1640 containing 10% v/v fetal calf serum (FCS), 15 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 0.1% w/v sodium bicarbonate with 100 IU/ml penicillin and 100 µg/ml streptomycin with the addition of 10 µg/ml PHA (Wellcome, Temple Hill, Dartford, England) for 72 h prior to co-culture. Fresh patient cells (10×10^6 cells) were then co-cultured with the PHA-activated donor PBMC (10×10^6 cells). Immediately on co-culture 2×10^6 of the mixed cell population were UV irradiated (254nm, 300µW/cm^2, 15sec), added back to the remaining cells and cultured for 29 days. After UV treatment cells were resuspended at 1×10^6 cells/ml in RPMI 1640 containing 10% v/v FCS, 15 mM HEPES, 0.1% w/v sodium bicarbonate, 25 µg/ml glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin, 2 µg/ml polybrene; 4µg/ml hydrocortisone (Sigma, St Louis, MO, USA), 20 U/ml interleukin 2 (Boehringer Mannheim, Germany) and 120 nU/ml anti-interferon (ICN

Biochemicals, Costa Mesa, CA, USA). Cells were maintained by half medium changes every 3 to 4 days after PHA stimulation, with the addition of fresh stimulated donor PBMC on days 7, 14 and 21. Virus production was assayed for by cell-free reverse transcriptase activity (Neate *et al*, 1987) or p24 activity (Abbott Diagnostics assay).

EXAMPLE 2

DNA Preparation and PCR Amplification

Non-pathogenic HIV-1 (e.g. strain C18) infected peripheral blood mononuclear cells (PBMC) were harvested 4 days after infection of phytohaemagglutinin (PHA) stimulated HIV-1 negative donor PBMC cultured by the method of Neate et al (1987) and washed in phosphate buffered saline (PBS). PBMC from Donor D36 and Recipients C18, C54 and C98 were prepared by Ficoll isopaque centrifugation of buffy coat cells and washed with PBS.

Approximately 10^7 cells were lysed in 1ml lysis solution (0.45% v/v NP40, 0.45% v/v Tween 20, 10mM Tris-HCl pH 8.3, 40mM KCl 2.5 mM $MgCl_2$) and digested with 60µg/ml proteinase K (Boehringer Mannheim) at 55°C for 1 hour followed by 100°C for 10 minutes. Lysates were stored at -20°C.

All polymerase chain reaction (PCR) primers (Table 1) and sequencing primers (Table 2) were synthesized using an Applied Biosystems model 391 DNA synthesizer using phosphoramidite chemistry.

Strict physical separation was maintained for sample, PCR reagent mix and PCR reaction preparations as well as amplification and analysis. Final reaction mixes (50µl) contained 2 µl neat or diluted cell lysate, 0.2µM each primer, 200mM dNTPs and 1.25 units Taq polymerase (Boehringer Mannheim) in PCR buffer, (10mM Tris-HCl pH8.3, 50mM KCl, 100µg/ml gelatine) adjusted to the optimum $MgCl_2$ concentration for the primer pair (1.5-3.0mM). Aliquoted reagent mix was overlaid with 50µl mineral oil prior to addition of DNA template lysate. After template denaturation at 94°C for 3 min amplification was achieved with 30 cycles f 94°C, 1 min; 55°C, 1 min; 72°C, 2 mins. A final elongation reaction was conducted at 72°C for 7 minutes. For double PCR

amplification 2 µl of first round product was added to the second reagent mix and amplified as before.

PCR amplified DNA was checked for quality, quantity and fragment size by agarose gel electrophoresis in Tris-Acetate-EDTA buffer (Sambrook et al, 1989) stained in ethidium bromide and viewed by UV transillumination.

EXAMPLE 3

DNA Sequence Analysis

The DNA sequence of PCR amplified HIV-1 regions was determined by the dideoxynucleotide method (Sanger et al, 1997) using Sequenase T7 polymerase (United States Biochemicals).

PCR amplified DNA was purified by PCR Magic prep resin chromatography (Promega). Approximately 2 to 7 µg purified DNA plus 10ng specific primer (Table 2) were denatured by boiling for 3 mins and snap frozen to -20°C. The initial labelling reaction was for 3 minutes at 22°C (room temperature) with ^{35}SdNTP (500Ci/mmol; Dupont) followed by dideoxynucleotide termination reactions at 37°C for 5 minutes. NP40, to 0.45% v/v, was included in denaturation and reaction mixes (Bachman et al, 1990). Sequencing reaction products were denatured in formamide and resolved on a 6% w/v polyacrylamide gel containing 8M urea, fixed in 10% v/v acetic acid, 10% v/v methanol and dried. Following autoradiography on XK1 film (Kodak) the gel sequences were read assembled, translated to protein and aligned using the PC/GENE suite of programs (IntelliGenetics, USA).

TABLE 1

PCR PRIMERS

PRIMER	SEQUENCE[1, 3]	POSITION[2]
CI-1	TGGAAGGGCTAATTTGGT(616)	1-18
CI-2	ATCTTCCCTAAAAAATTAGCCTGTC(617)	2099-2075
LTR-3'	AGGCTCAGATCTGGTCTAAC(618)	9559-9540
SK68	AGCAGCAGGAAGCACTATGG(619)	7786-7805
CI-6	TGCTAGAGATTTTCCACAC(620)	9709-9691
KS-2	AGTGAATAGAGTTAGGCAGG(621)	8326-8345
RT5'-v3	GTAAGACAGTATGATCAGATA(622)	2418-2438
RT3'-v2	TTGTAGGGAATTCCAAATTCC(623)	4660-4640
RT5'-v2	CAGGATCCTACACCTGTCAACATAAT(624)	2487-2506
RT3'-v1	GGGAATTCCTTATTCCTGCTTG(625)	4655-4634

1. Sequence is presented from 5' to 3' of the primer.
2. Position is according to the numbering of HIV-1 in Myers *et al* (1992).
3. SEQ ID NOs are given in parentheses.

TABLE 2

SEQUENCING PRIMERS

PRIMER	SEQUENCE[1, 3]	POSITION[2]
KS3	CCAGAAGTTCCACAATCC(626)	8570-8553
KS4	TTCTTCTAGGTATGTGGAG(627)	8753-8735
KS5	AGTGAATTAGCCCTTCCAG(628)	9093-9075
KS6	TGCTAGAGATTTTCCACAC(629)	9709-9691
SP2	TGCTCTGGAAAACTCAT(630)	8006-8022
SP3	CTTTCTATAGTGAATAGAG(631)	8318-8336
SP4	TATTGGAGTCAGGAACT(632)	8618-8634
SPR	GGTCTAACCAGAGAGAC(633)	9547-9531

1. Sequence is presented from 5' to 3' of the primer.
2. Position is according to the numbering of HIV-1 in Myers *et al* (1992).
3. SEQ ID NOs are given in parentheses.

EXAMPLE 4

Cells and Cell Culture

Peripheral blood was obtained from HIV-1 sero-negative volunteers and mononuclear cells prepared by centrifugation on a Ficoll/Hypaque density gradient (Peper et al, 1968). PBMC were activated with phytohemagglutinin (PHA; 10μg/10^6 cells) for 48 h at 37°C washed and then cultured in RPMI 1640 medium containing 10% v/v heat inactivated foetal calf serum, 15mM HEPES, 0.1% v/v sodium bicarbonate, 25μg/ml polybrene (Sigma), 10% v/v interleukin 2 (Boehringer Mannheim) and 1:1000 anti-interferon (Miles) (IL-2 medium). Non-PHA stimulated cells were prepared in a similar manner except they were cultured in medium lacking PHA and IL-2.

EXAMPLE 5

Antipeptide-antisera

Antibodies specific for HIV-1 Nef were raised against a peptide corresponding to the predicted amino acid residues 15-27 (AVRERMRRAEPAA SEQ ID NO: 634) of Nef encoded by the HIV-1 clone pNL4.3 (Kemp et al, 1988). The peptide was conjugated to keyhole limpet hemocyanin (KLH; Calbiochem, Behring Diagnostics, CA) *via* glutaraldehyde and this complex used to immunise sheep (0.5mg peptide conjugate/sheep). Antibodies to the peptide were purified by affinity chromatography. Reactivity of the antibodies with recombinant HIV-1 Nef 25 and 27 was demonstrated by immunoblotting.

EXAMPLE 6

Reactivity of anti-Nef $_{(15-27)}$ with HIV C18-infected Cells in Immunoblotting

Seven days post-infection HIV-1 C18-infected PBMCs and mock - infected cells were washed in PBS then lysed (0.5% w/v NP-40, 0.5% w/v sodium deoxycholate, 50mM NaCl, 25mM Tris-HCl, 10mM EDTA, 0.01% w/v sodium azide and 10mM phenylmethylsulphonylfluoride). After nuclei were spun out lysates were electrophoresed in a 13% w/v SDS-polyacrylamide gel (SDS-PAGE) and subsequently transferred to Hybond-C nitrocellulose (Amersham, Buckinghamshire, England) for 1 h at 100 V using a Bio-Rad protein transfer cell (Bio-Rad, Richmond, Ca). Membranes were pre-incubated with 1% w/v BSA/PBS for 2 h at room temperature and then reacted with affinity purified sheep anti-Nef$_{(15-27)}$, diluted 1:100, overnight at room temperature. After three washes in 1% w/v BSA/PBS, the blots were incubated with donkey anti-sheep Ig conjugated to biotin (Amersham, diluted 1:500) for 1 h at room temperature. After extensive washing as described above the membranes were incubated with streptavidin-conjugated horse radish peroxidase (Amersham; diluted 1:500 for 1 h at room temperature. All dilutions were made with 1% w/v BSA in PBS. After further washing the membrane was developed with phenylenediamine substrate (Dako, Dapopatts, Denmark). The antibody preparation used in the immunoblotting experiments was free of detectable antibodies to the immunogenic carrier protein and coupling reagent.

EXAMPLE 7

Analysis by Polymerase Chain Reaction Amplification

A 5' fragment defined by primers Cl-1 and Cl-2 containing the 5' LTR and part of the *gag* gene was amplified. DNA from HIV-1 C18 infected PBMC gave an amplified fragment (amplimer) of about 1.9 kb compared with 2.1kb for pHXB2 control template, implying a deletion of about 200bp from HIV C18. Further amplification of this fragment with primers defining the U3 region of the LTR (Cl-1 and LTR-3') gave amplimers of about 300 bp for HIV-1 C18 infected PBMC DNA compared with 340bp for C18 and D36 PBMC DNA and 484 bp for pHXB2 control. This implies the loss f approximately 140 to 180bp from the U3 region of these proviral DNAs.

To analyse the *nef*-gene-3' LTR region, the nested primer pairs SK68-Cl-6 and KS-2-LTR-3' were used in a double PCR. Amplimers of approximately 830bp were obtained for HIV-1 C18 infected PBMC DNA as well as for PBMC DNA from Donor D36 and Recipients C18, C54 and C98 compared with approximately 1230bp for pHXB2 DNA. These results suggest that about 400bp of DNA have been deleted from the Donor and Recipient proviral DNAs.

In comparison, amplification of the polymerase gene region by double PCR with the nested primer pairs RT5'-v3-RT3'-v2 and RT5'-v2-RT3'-v1 gave a fragment (approximately 2.1 kb) the same size as the molecular clone pHXB2 fragment for HIV-1 C18 infected PBMC DNA, suggesting that deletions from this region were unlikely.

EXAMPLE 8

Nucleotide Sequence of the nef-3' LTR Region

PCR amplification experiments indicated an approximately 200bp nucleotide deletion from both the *nef* gene and LTR regions of Donor D36 PBMC and Recipient C18 HIV-1 proviral DNA. To further analyse these regions, the DNA sequence was determined for the PCR amplified *nef-3'* LTR region of D36 PBMC, C18 isolates HIV_{MBC} and HIV_{SIV} as well as isolate C98 HIV infected PBMC proviral DNA. The 3' region was amplified with outer primers (SK68-Cl6) and inner primers (SK68-LTR 3' or KS2-Cl6) and sequenced directly using a number f internal sequencing primers based on the HIV-

1_{NL43} nucleotide sequence (Table 2).

Alignment of the nucleotide sequences of the amplified 3' region of donor D36 PBMC and recipient C18 isolates HIV_{MBC} and HIV_{StV} and C98 HIV (Fig 1) showed a number of nucleotide sequences changes, including deletions, relative to the nucleotide sequence of wild-type infectious HIV-1 (HIV-1_{NL43}). In the region of alignment, D36 PBMC lacked 291 nucleotides, C18 HIV_{StV} differed in size by 388 nucleotides (comprising deletions of 397 nucleotides and an insertion of 9 nucleotides), C18 HIV_{MBC} differed by 456 nucleotides and C98 HIV lacked 158 nucleotides compared with HIV-1_{NL43}. The overall identity with HIV-1_{NL43} nucleotide sequence of D36 PBMC, C18 HIV_{StV}, HIV_{MBC} and C98 HIV nucleotide sequences, including deletions, was 73% (1157/1596), 67% (1459/1592), 62% (982/1592) and 79% (1105/1399), respectively.

The D36 PBMC sequence differed from HIV-1_{NL43} in a number of features. A change in the wild type *tat* termination codon from TAG to TCG (Ser) extended the third *tat* exon (which starts at splice acceptor 10) by a further 15 amino acids to terminate at a conserved TAG (Fig 1). The resulting C-terminal peptide is rich in charged amino acids (8/15) (Fig 2a). The wild type *rev* termination codon has also changed (TAG to GAG, Glu) and the third *rev* exon is extended for 14 codons to terminate at a conserved TAG (Fig 2b). The encoded extra amino acids are mainly polar (11/14) and charged in nature (Fig 2b). The sequence also encodes the C-terminal 237 amino acids of Env gp41 (Fig 3) terminating at the normal termination codon. The D36 PBMC Env amino acid sequence has 85% identity with the HIV-1_{NL43} sequence, increasing to 89% if similarities are included.

There are significant differences from HIV-1_{NL43} downstream of the *env* (gp41) gene. A change in the fifth *nef* codon, from TGG (Trp) to TGA (Fig 1), introduces an early termination in the D36 PBMC *nef* gene. The encoded Nef protein is identical to the N-terminal 4 amino acids of HIV-1_{NL43} Nef (Fig 4). Following the early termination there are deletions of 33, 47, 93 and 91 nucleotides and a region of low sequence homology, compared with HIV-1, prior to the wild type *nef* termination codon site (HIV-1 nts 9405-9407). As well as removing a significant part of the *nef* gene, these deletions also

bring into phase a further 6 termination codons. Whil the polypurine tract (plus strand primer binding site) and the first 38 nucleotides of the LTR U3 region are perfectly conserved, downstream U3 region sequences are disrupted by the 93 and 91 nucleotide deletions and the low homology region. The resulting U3 region lacks recognition sequences for the transcription factors *c-myb*, USF and TCF1α as well as one of the suggested NF-AT sites (Gaynor et al, 1992). Downstream from the 91 nucleotide deletion, a 59 nucleotide region of low homology contains two extra NFKB enhancer sites 19 nucleotides upstream of the usual site of a pair of NFKB sites, the upstream one of which is altered in its 5'-half in D36. Sequences further downstream are highly conserved with respect to HIV-1_{NL43}, including the position and number of Sp1 basal promoter sites, TATA box, TAR and polyadenylation signal sequences.

Similar to D36 PBMC, the C18 HIV_{StV} and HIV_{MBC} sequences show the *tat* third exon to be extended by 15 codons. All but two codons (altered by point mutations) are identical to those of D36 PBMC (Fig 2a). The *rev* third exon of both C18 isolates is also extended (Fig 2b) but by only three codons, identical to the first three codons of the D36 PBMC *rev* extension. The same 237 amino acid coding region of Env gp41 is found in both the C18 HIV DNA sequences (Fig 3) and shows 85% identify, increasing to 88% if similarities are included, with the same region of the HIV-1_{NL43} Env gp41.

It is in the *nef* gene and LTR regions that the major differences from wild-type HIV-1 arise, just as in D36 PBMC. The *nef* gene of C18 HIV_{StV} encodes 24 amino acids with 9 of the 10 N-terminal being identical to the HIV-1_{NL43} Nef protein (Fig 4). Thereafter, deletions of 177 and 11 nucleotides cause a frameshift and termination at the 25th codon (Fig 1). Downstream deletions of 120, 82 and 7 nucleotides cause further loss of wild type *nef* gene sequence and bring into phase a further three termination codons.

The *nef* gene of C18 HIV_{MBC} encodes only 7 amino acids with only the initiator methionine identical to the HIV-1_{NL43} Nef protein. This loss of identity and early termination is brought about by a 250 nucleotide deletion after the fifth nucleotide of the *nef* gene. Downstream deletions of 120 and 86 nucleotides cause further loss of wild-type *nef* gene sequences. In both C18 isolates there is perfect conservation of the

polypurine tract and 29/31 nucleotides at the 5' end of the U3 region immediately before the 120 nucleotide deletion (Fig 1). This deletion together with the downstream 82 and 7 nucleotide deletions in HIV_{StV} and 86 nucleotide deletion in HIV_{MBC} and the low homology region cause the loss of the 5' half of the NRT-1 site (Yamamoto et al 1992) and the downstream NFAT site. A third NFKB site is present 31 (HIV_{StV}) and 33 (HIV_{MBC}) nucleotides upstream of the expected pair of NFKB sites which are themselves separated by 13 nucleotides instead of the 4 nucleotides in HIV-1_{NL43}. The 5'-most Sp1 site sequence is slightly altered but sequences downstream including the other 2 Sp1 sites, the TATA box, TAR and polyadenylation signal sequences are identical to HIV-1_{NL43} sequence.

The three sequences, D36 PBMC, C18 HIV_{StV} and C18 HIV_{MBC} show a number f similarities consistent with the transmission of virus from person D36 to person C18 as well as a number of differences indicating post-transmission divergence of sequence. All three have *tat* open reading frames (ORFs) extended by 15 codons. All three have extended *rev* ORFs. The new *rev* termination codon in both C18 HIV-1 isolates, three codons downstream of the HIV-1_{NL43} *rev* termination codon, has a point mutation in D36 PBMC to make a Glu codon so that it continues for a further 11 codons (Fig 2b) to terminate at a conserved TGA. The partial Env gp41 amino acid sequences are more closely related to each other (86% identity or 90% including similarities) than to HIV-1 (85% and 89%, respectively).

The nucleotide sequence of the *nef* and LTR region of the HIV-1 isolate from recipient C98 (C98 HIV) is 90.3% identical (1264/1399) to the HIV-1 sequence, ignoring deletions. Similar to the D36 PBMC and C18 HIV_{StV} and HIV_{MBC} isolates the C98 HIV sequence shows the third exon of *tat* to be extended by 15 codons with all but one being identical to the D36 PBMC *tat* extension. Also, the *rev* gene is extended by 3 codons, 2 of which are identical to the first 2 codons of the D36 PBMC *rev* extension. The sequence also encodes the C-terminal 223 amino acids of Env gp41 terminating at the normal termination codon. The C98 HIV Env amino acid sequence has 89% identity with HIV-1 Env sequence, increasing to 92% of similarities are included.

As with the D36 PBMC and the C98 HIV isolate sequences it is the *nef* gene and LTR regions that major differences from the HIV-1 sequence arise. The *nef* gene open reading frame of C98 HIV is much longer than in D36 PBMC, C18 HIV_{SfV} and HIV_{MBC}, encoding 85 amino acids compared with 206 amino acids for HIV-1_{NL43}. Sixty eight of those 85 amino acids are identical to the N-terminal sequence of HIV-1_{NL43} Nef. The single, small deletion (16 nucleotides) in the C98 HIV *nef*-alone regions (Table 3) occurs after *nef* codon 82 causing a frameshift and termination after a further 3 codons at the start of the highly conserved polypurine tract sequence immediately before the 3'-LTR. The *nef*/LTR region has two deletions totalling 142 nucleotides. The 5'-most deletion of 42 nucleotides includes the splice acceptor 12 sequence. The NRT-1, dyad symmetry and *myb* response element sequences are all intact. However, the downstream 100 nucleotide deletion includes sequences from the 3' end of the 5'-NF-AT and all of the 3' NF-AT sequences as well as the USF transcription factor recognition sequence. The downstream low homology region of 77 nucleotides lacks the TCF-1α sequence but has two additional NFKB sites 13 nucleotides apart and 26 nucleotides upstream of the 3'-half-remmant of the normal 5'-NFKB site. Sequence downstream, including the 3'-NFKB site, the 3 Sp1 sites, TATA box TAR and polyadenylation signal sequences are all highly conserved.

The main feature of the sequences is the series of deletions, with respect to HIV-1, in the *nef* gene-3'-LTR region. These can be grouped into two regions namely the *nef*-alone region, that part of the *nef* gene upstream of the LTR, and the *nef*/LTR region, where the *nef* gene and LTR U3 regions overlap. The deletions in these regions of each of the sequences start and end at the same or similar positions (Table 3). The deletions are larger in C18 HIV_{SfV} and C18 HIV_{MBC} sequences where totals of 397 and 456 nucleotides have been deleted (relative to HIV-1_{NL43}) compared to 291 nucleotides, from D36 and 158 nucleotides from C98 HIV. In the *nef*-alone region the two deletions in C18 HIV_{SfV} and the single deletion in C18 HIV_{MBC} occupy the same region as the three deletions in D36 PBMC. Similarly, the *nef*/LTR region in the three deletions in the C18 HIV_{SfV}, the two deletions in the C18 HIV_{MBC} and the D36 PBMC sequences occupy the same region. These findings indicate that mutant virus was transmitted from D36 to C18 after which further deletions and rearrangements occurred. Similarly, the

sequence f C98 HIV in the *nef*/LTR region indicates two deletions occupying the same region as the *nef*/LTR deletions in D36 and the C18 sequences. However, the size (only 16 nucleotides) and the position of the deletion in the *nef*-alone region of C98 HIV are distinct from those of the D36 PBMC and C18 sequences.

The timing of transmission of virus by transfusion was that recipient C18 was transfused approximately 19 months after C98. Consistent with the relative timing of transmission and the sequence similarities and differences is the suggestion that at the time of transmission to C98, the D36 sequence had deletions in the *nef*/LTR region but not in *nef*-alone region. After transmission to C98, the C98 virus developed further deletions and rearrangements, including the deletion in the *nef*-alone region. The D36 virus evolved so that at the time of transmission to C18, further deletions and rearrangements had occurred including deletion of sequence from the *nef*-alone region distinct from the C98 HIV *nef*-alone region deletion. After transmission to C18, further deletions and rearrangements occurred in the C18 virus giving rise to at least two sequences (HIV_{SkV} and HIV_{MBC}).

The *nef*-alone deletion region may be a mutation or recombination "hotspot" as it includes sequences that were found to be variably duplicated in 28 out of 54 Nef protein sequences derived from 8 of 12 patients analysed in a study (Shugars *et al* 1993). The sequence between the *nef*-alone and the *nef*/LTR region deletions is highly conserved and is important in provirus integration into the infected cell genome and interacts with a number of cellular proteins. It is interesting that the sequence equivalent to HIV-1_{NL43} nucleotides 9209 to 9225 is retained in D36 and C98 HIV but lost in the C18 HIV sequences. This includes part of a sequence of dyad symmetry (9210 to 9231) and is a significant part of the binding site for NRT-1 (Yamamoto et al 1991) which has been shown to have a negative regulatory effect on HIV-1 expression. The presence f this sequence in D36 and C98 HIV and its absence from the C18 isolates may correlate with the inability to isolate virus from D36 PBMC and the poor replication of C98 HIV but the ability to isolate HIV-1 from C18 PBMC. The deletion of sequence equivalent to nucleotides 9281 to 9395 f HIV-1_{NL43} causes the loss of some transcription factor binding sites including NFAT and USF from the D36, C18 HIV and C98 HIV sequences.

A further similarity between the D36, C18 HIV_{SIV}, C18 HIV_{MBC} and C98 HIV sequences is a region of low homology to $HIV\text{-}1_{NL43}$ extending downstream of the *nef*/LTR deleted region to the NFKB enhancer/Sp1 promoter site region. This low homology region in fact consists of incomplete duplications of part of the NFKB/Sp1 region (Fig 5) resulting in D36 and C98 HIV having 2 extra NFKB sites upstream of an altered 5' NFKB site while the C18 sequences have one extra NFKB site and altered spacing between the 5' and 3' wild type NFKB sites due to an insertion of 9 nucleotides.

For the C18 and C98 HIV-1 isolates virus replication was assessed in PHA-stimulated and non-stimulated PBMCs (Figs 6 and 7). In PHA-stimulated PBMCs we also studied cell surface CD4 and IL-2R expression (Fig 8). In comparison with HIV-1 wild-type SI and NSI isolates clearly both C18 HIV_{MBC} and C98 viruses are replication competent, though C98 HIV replicates more poorly than C18 HIV_{MBC} and are of the NSI phenotype when syncytium formation and CD4 and IL-2R surface expression are taken into account. Additionally, and more surprisingly, these two viruses replicated almost as efficiently in non-PHA stimulated PBMCs when compared to a typical local wild type SI isolate (HIV-1 228200, Fig 7).

When protein expression was assessed for C18 HIV_{MBC} and C98 HIV_{MBC} although structural proteins were identified, no typical Nef protein was seen in infected cells. However, analysis of cell lysates prepared from PBMC infected with C18 HIV_{MBC} or PBMC infected with C98 HIV_{MBC} (which were subsequently stimulated by UV irradiation, see Valerie *et al*, 1988) by Western immunoblotting using two antibodies specific for the N-terminal region of Nef showed the presence of smaller proteins of 19 kDa and 21 kDa, respectively. These proteins were not observed in mock-injected control PBMC lysates and were not observed when the infected-cell lysates were probed were probed with antibodies reactive with the C-terminus of Nef.

Thus, although the C18 and C98 HIV isolates are replication competent *in vitro* they clearly replicate differently using different conditions for cell activation and from the known functions f HIV-1 Nef protein and the LTR show that the major deleti n in the *nef* gene and/or the LTR is at least in part responsible for the outcome f infecti n,

implicating the importance f Nef and/or th LTR in the clinical outcome f infection *in vivo*.

EXAMPLE 9

Determination of Degree of Relatedness Between Viruses

To determine the degree of relatedness between viruses such as between mutants or between mutants and a wild-type virus and to ascertain putative infected patients, the method of Delwart *et al* was employed.

EXAMPLE 10

Immune responsiveness of subjects infected by non-pathogenic HIV-1 isolate

In this example, the donor and recipients of the cohort were tissue typed and assessed for basic cellular immune responses. Proliferative responses and IL-2 production to the mitogens ConA and PHA, to allogeneic mononuclear cells (irradiated pooled mononuclear cells from 20 random donors) and to recall antigens (e.g. influenza and tetanus toxoid) were within normal ranges. While at the immunogenetic level, HLA typing failed to identify a consistently common allele or haplotype within the group.

The conservation of CD4+ counts observed in the cohort, the relative integrity of their immune system and the varied HLA types of the donor and recipients further supports the fact that the symptomless condition of the cohort members is due to a non-pathogenic strain of HIV-1 or a strain of low virulence.

Accordingly, this provides a screening procedure for subjects putatively infected by a non-pathogenic HIV-1 isolate where such subjects are seropositive for HIV-1 (e.g. have antibodies to an HIV-1 glycoprotein) yet have normal proliferative responses and cytokine production to mitogens, allogeneic mononuclear cells and to recall antigens.

EXAMPLE 11

Clinical Immunology of Cohort

To establish that the donor and the recipients belonging to the cohort exhibit normal immunological profiles, members of the cohort were assayed for CD3, CD4, CD8, lymphocyte count, CD4/CD8 ratio and β-2-microglobulin over time since seroconversion.

Parameters considered normal in non-infected individuals range as follows:

Parameter		
CD3	55-82%	620-2200 (x10^6/L)
CD4	29-58%	420-1410 (x10^6/L)
CD8	12-43%	200-980 (x10^6/L)
Lymphocyte count	1000-3500 (x10^6/L)	
CD4/CD8	0.7-3.7	
β-2-microglobulin	0.00-2.20 mg/L	

The results are shown in Figures 10(a)-(g) and clearly show that the immunological profiles of cohort members are substantially normal further highlighting the non-pathogenicity of the HIV-1 isolates of the present invention. Figure 10(g) shows a graph of the Kaplan-Meier (Ox and Oates, 1989) estimates of time to disease progression (AIDS or CD4 > 250). The results demonstrate that the difference is large in spite of the conservative bias, with a median time to progression of 6.2 years in the main database. An exact logrank test (Cytel Software Corporation, 1989, StatXact: Statistical Software for Exact Nonparametric Inference. Cambridge, Massachusetts.) was performed, demonstrating that the difference between the groups was highly statistically significant (logrank statistic 11.8, $p<0.0001$).

Table 3

Deletions and their sizes in the *nef*-alone and the *nef*/LTR regions of the Long-Term Asymptomatic HIV-1 Sequences

Sequence	*nef*-alone Region	Region Deletion (nt)	*nef*/LTR Region	Region Deletion (nt)	Total Deletion (nt)
D36 PBMC	8830-8862 (33) 8882-8928 (47) 9009-9035 (27)	107	9112-9204 (93) 9281-9371 (91)	184	291
C18 HIV_{SIV}	8830-9006 (177) 9019-9029 (11)	188	9105-9224 (120) 9281-9362 (82)	202	390
C18 HIV_{MBC}	8792-9041 (250)	250	9105-9224 (120) 9281-9366 (86)	206	456
C98 HIV	9033-9048 (16)	16	9148-9189 (42) 9271-9370 (100)	142	158
C54 PBMC	incomplete	?	9281-9375 (95)	95	95 +

Sequence numbering relates to the equivalent position in HIV-1 NL4-3. Numbers in brackets are the deletion sizes in nucleotides (nt).
The *nef* ORF starts at nt 8787and the 3'-LTR starts at nt 9074 in HIV-1 NL4-3.

EXAMPLE 12

Sequencing of isolate HIV-1 C18$_{MBC}$

The genome of variant HIV-1 designated C18 HIV-1$_{MBC}$ was amplified by the polymerase chain reaction (PCR) as 7 overlapping fragments using the sets of inner and outer oligonucleotide primers, designed using the programme PCRPLAN (IntelliGenetics), listed in Table 5 and either UlTma (Applied Biosystems) or a mixture of KlenTaq and Pfu (KlenTaq LA, Ab Peptides Inc) polymerases (for faithful amplification of long fragments). The resulting fragments were cloned into the SmaI site of the plasmid vector pGEM7Zf+. Insert-containing clones representing each region of the full length variant HIV-1 were sequenced by a nested deletion strategy (Gou & Wu, 1982) and cycle sequencing with Taq polymerase and dye labelled primers complimentary to the T7 or SP6 sites within the cloning vector. Nucleotide sequences were entered and collated by ASSEMGEL and SEQIN (IntelliGenetics) and SEQED (Applied Biosystems) and translated to the encoded amino acid sequences using TRANSL (IntelliGenetics) programmes. Sequence alignments used NALIGN, CLUSTAL (IntelliGenetics) and SEQED programmes.

The full length sequence (Fig 9; SEQ ID NO:800) of isolate HIV-1 C18$_{MBC}$ is 9207 nucleotides long which is 506 nucleotides shorter than the HIV-1 sequence. This size difference is comprised of 126 nucleotides of insertions and 632 nucleotides of deletions, see Table 6. The most extensive differences between the HIV-1 C18$_{MBC}$ sequence and HIV-1$_{NL43}$ are in the U3 region of the LTR and in the *nef* gene, as hereinafter described.

The 5' LTR has deletions of 120 and 87 nucleotides and a region of low sequence homology, which is the result of an imperfect duplication of the downstream NFκB and Sp1 response sequences. These result in the loss of sequence from a number sites important in the regulation of transcription of HIV-1 genes, including the negative response element (NRE) and the response elements for a number of transcription factors including NF-AT, NRT-1, USF and TCF-1α. Furthermore, the low homology region contains an extra NFκB and Sp1 sites as well as an insertion of 9 nucleotides between

the usual NFκB sites. Downstream f the NFκB sites the sequence of the LTR has a high level of homology (96.2%) with the same region of HIV-1.

The *gag* gene contains 3 insertions, which represent direct repetitions of adjacent sequences. The first is a perfect repeat of 15 nucleotides after the equivalent of nucleotide 1134 of HIV-1_{NL43} and adds 5 amino acids to the C-terminus region of $p17^{gag}$. The remaining 2 insertions are imperfect and perfect repeats of 30 and 6 nucleotides, respectively, after the equivalent of HIV-1 nucleotides 2163 and 2232, respectively. These encode an extra 12 amino acids in the C-terminus region of $p15^{gag}$ just downstream of the *gag* to *pol* frameshift sequences. The variation in sequence length of the *gag* gene at these two positions is unusual. The homology of the encoded amino acid sequence of HIV-1 $C18_{MBC}$ and HIV-1 for the *gag* p17, p24, and p15 proteins is 87.1%, 93.5% and 94.3%, respectively.

In the *pol* ORF, the encoded proteins have high homology with the HIV-1_{NL43} sequences being 95.5% overall comprising p10 protease 92.9%, p66 reverse transcriptase 95.4% and p34 integrase 95.8%. The amino acid sequence of the p61 RT lacks the mutations associated with resistance to the nucleoside (AZT, ddI, ddC)and non-nucleoside (Nevirapine) analogue drugs used in the treatment of HIV-1-infected persons.

The *vif* gene encodes a 192 amino acid protein with 88.0% homology with that of HIV-1. The *vpr* gene encodes a 96 amino acid protein with 89.6% homology with that f HIV-1_{NL43}.

There are 2 insertions and 1 deletion of sequences in the *vpu* gene. The insertions of 3 and 9 nucleotides are after the equivalent of nucleotide 6071 and 6234, respectively, of HIV-1_{NL43}. These add 1 amino acid after amino acid 3, and 3 amino acids after amino acid 59 of the encoded *Vpu* protein. The deletion of 12 nucleotides after the equivalent of HIV-1_{NL43} nucleotide 6261 deletes 4 amino acids from the C-terminal region of *Vpu* as well as from the signal peptide of the *env* polyprotein, which is encoded by an overlapping reading frame. Amino acid sequence homology of HIV-1 $C18_{MBC}$ Vpu with HIV-1_{NL43} is 85.2%.

The sequence encoding the *env* gp120 has 9 insertions totalling 45 nucleotides (encoding 15 amino acids) and the deletion of a total of 18 nucleotides (encoding 6 amino acids). These are listed in Table 6. All of these events (insertions and deletions) are at positions in the *env* gene. This is within the *env* V3 coding region, immediately upstream of the sequence encoding the so called V3 tip (or loop) amino acid sequence, Gly Pro Gly Arg. The V3 region sequence is that of a typical clade B subtype (North America, Europe and Australia) being identical to the clade B consensus sequence (based on 186 *env* sequences) at 29/35 positions. The type of amino acid at positions 11 and 28 of the V3 loop region (where position 1 is the Cys at amino acid 266 of the *env* gp120) is predictive of the viral non-syncytium / syncytium forming phenotype (Fouchier et al, 1992). The HIV-1 $C18_{MBC}$ *env* gene encoded amino acid sequence has Ser at position 11 and Ile at position 28 of the V3 loop region. The lack of a positively charged amino acid at both positions is strongly indicative of a non-syncytium viral phenotype. The overall amino acid sequence homology with HIV-1_{NL43} (ignoring deletions and insertions) is 86.1%, comprising 85.5% for the gp120 region and 87.6% for the gp41 region.

Both the *tat* and *rev* second exon open reading frames (ORF) are longer than in HIV-1_{NL43}. A change of the *tat* termination codon from TAG to TCG extends the *tat* ORF to a downstream in phase termination codon extending the encoded *tat* amino acid sequence by 15 residues, compared with the 86 amino acid long HIV-1_{NL43} *tat* protein, to a total length of 101 amino acids. However, this is the usual length of the HIV-1 *tat* protein.

Similarly, the normal *rev* termination codon is changed from TAG to GAG. This extends the *rev* ORF to an in-phase termination codon 3 codons downstream so that the encoded Rev protein is 119 amino acids long instead of the usual 116.

As mentioned above the most extensive differences between the sequences of the isolate HIV-1 $C18_{MBC}$ and HIV-1_{NL43} are in the *nef* gene and the LTR region. While the *nef* gene overlaps the 3' LTR, these differences are found in both the *nef* alone and the *nef* / LTR overlap regi ns. The HIV $C18_{MBC}$-encoded *nef* protein is only 24 amino acids

long compared with the normal length of 206 amino acids. This severe shortening of the *nef* protein is due to the deletion of 188 nucleotides (the 177 and 11 nucleotide deletions) from the *nef*-alone region which also brings into phase a termination codon, TAG, at the resulting 25th codon. Downstream there is further loss of potential *nef* gene sequences by the 120 and 87 nucleotide deletions situated in the *nef* / LTR overlap region. The resulting 24 amino acid *nef* protein is identical to the N-terminus of the HIV-1_{NL43} *nef* at 9 of the first 10 positions. Thereafter, homology is lost completely.

Some sequences used in the generation of mature mRNAs are altered or lost in $C18_{MBC}$. The dinucleotide immediately after the splice donor site 2 (SD2) at nts 4818-4819 (HIV-1_{NL43} equivalent nts 4963-4964) is changed from the conserved GT to GC. It is expected that this change would lead to loss of function of this site as a splice donor. Splice donor 2 is used in the processing of HIV-1 transcripts to some of the mRNAs that encode Tat, Rev and *nef* proteins. Similarly the splice acceptor site 7 (SA7) sequence at nts 6477-6478 (HIV-1_{NL43} equivalent nts 6602-6603) is changed from the conserved AG dinucleotide to TC. This change is expected to lead to loss of function of this site as a splice acceptor. While this SA site is used in HIV-1 mRNA processing it is not a major site and is not used in the production of the regulatory proteins (Tat, Rev or *nef*) mRNAs. The splice donor 12 site is absent from the $C18_{MBC}$ sequence (NL43 equivalent nts 9161-9162) as it is within the first deletion region in the *nef* / LTR overlap region which occurs at nt 8797 and results in the loss of NL43 nucleotides 9105 to 9224. It is significant that the SA12 site is absent from the sequence of all of the cohort virus isolates so far obtained as well as from the sequence of D36 PBMC, however, the C54 PBMC sequence does contain the SA12 site. SA12 is not used in the processing of mRNAs that encode the viral regulatory proteins. Normally SA12 is used in splicing in conjunction with SD1, 2, 3 and 4 and the resulting spliced RNA is probably not a mRNA but may have a regulatory role involving binding to cellular proteins (Smith et al, 1992).

An interesting feature of the sequence of the HIV-1 $C18_{MBC}$ isolate is the deletion and rearrangement of sequence from the 5'-LTR U3 region and the deletion f sequence from the *nef* gene (both *nef* alone and *nef* / 3' LTR regions). These being the nly

features of the sequence distinct from disease-causing HIV-1. The lack of AIDS or AIDS-like symptoms in the patient C18 is attributed to the effects of the loss of LTR sequence and / or the loss of *nef* coding sequences and their role in the pathogenesis of AIDS.

TABLE 4

Primers used to Amplify Overlapping regions of HIV-1 $C18_{MBC}$

Primer	5'-Coordinate	Direction (+/-)	Primer Length (nt)	Sequence
CL 1A	1	+	30	TGGAAGGGCTAATTTACTCCCAAAAAAGAC
CL 14	896	-	25	AATCGTTCTAGCTCCCTGCTTGCCC
CL 1B	1	+	30	AATCCCGGGTGGAAGGGCTAATTTACTCCC
CL 13	796	-	31	CCTCTAGACCGCTTAATACTGACGCTCTCGC
CL 11	682	+	23	TCTCTCGACGCAGGACTCGGCTT
CL 18	3440	-	30	CTGTTTTCTGGCAGTTCTAGCTCTGCTTCT
CL 12A	732	+	26	TTACCCGGGCGGCGACTGGTGAGTAC
CL 17	3330	-	32	CCCTCTAGACTTGCCCAATTCAATTTTCCCAC
CL 26	3193	+	39	GCACACCAGAGAAAAAGCATCTGGAAGAACCCCCATTCC
CL 6B	9671	-	39	TGCTAGAGATTTTCCACACGGACTAAAAGGGTCTGAGGG
CL 27	3251	+	39	CCATCCTGATAAATGGACAGTACAACCCATAGTACTGCC
CL 28	639	-	37	TGGCCCAAACATTATGTACCTCTGCATCATATGC
CL 19	5448	+	30	AGCAGGACATAACAAGGTAGGATCTCTACA
CL 24	8422	-	28	GGATCTGTCTCTGTCTCTCTCTCCACCT

Underlined sequences depict added restriction enzyme site

+ and - orientations refer to sense and antisense strands of the double stranded DNA sequence

TABLE 5

Sequence Deletions and Insertions in HIV-1 $C18_{MBC}$ Compared with HIV-1_{NL43}

Gene or Region	Position (nt) $C18_{MBC}$	Position (nt) NL43	Deletions (nt)	Insertions (nt)
5'-LTR U3	29	29	120	-
5'-LTR U3	85	205	87	-
5'-LTR U3	154	360	-	9
gag p17	939	1134	-	15
gag p15	1982	2163	-	30
gag p15	2081	2232	-	6
vpu	5927	6062	-	3
vpu/env	6092	6234	-	9
vpu/env	6128	6261	12	-
env	6483	6628	-	6
env	6514	6653	2	-
env	6524	6665	1	-
env	6630	6772	-	9
env	6646	6778	-	3
env	7011	7141	6	-
env	7140	7276	3	-
env	7195	7334	-	6
env	7266	7399	3	-
env	7278	7414	-	6
env	7290	7420	-	2
env	7300	7429	-	1
env	7314	7441	3	-
env	7463	7593	-	3
env	7471	7598	-	9
nef	8711	8829	177	-
nef	8723	9018	11	-
nef / LTR	8798	9104	130	-
nef / LTR	8854	9280	87	-
LTR U3	8923	9435	-	9
			632	126

EXAMPLE 13

Macrophage Isolates of HIV-1 C18 and HIV-1 C98

HIV-1 has been isolated from the macrophages of patients C18 and C98.

Patient monocytes were prepared as follows. Whole blood was spun at 2000rpm for 10 minutes. Plasma was removed into a separate tube and the remaining cells were diluted 1:2 in PBS$^-$ (magnesium and calcium free phosphate buffered saline). This was underlaid with 10 ml of Ficoll Isopaque and spun at 2000rpm for 20 minutes. Cells were collected from the interphase and washed three times with PBS$^-$. These cells were then seeded into a 6 well Costar tray at a concentration of 1.0×10^7/ml and allowed to adhere for 1 hour. Any non-adherent cells were removed by aspiration.

Donor HIV-1 negative macrophages for use in co-cultivation were prepared as follows. Peripheral blood mononuclear cells were purified from whole blood using Ficoll/Isopaque density gradient. These cells were seeded at a concentration of 2.0×10^6/ml in teflon. PBMC were cultured in the presence of 3μg/ml of PHA and 1000U/ml of M-CSF 3 days prior to co-culture.

On day of co-culture, donor PBMC were CD8 depleted. Dyna beads coated with anti-CD8 were used for this purpose. Dyna beads were washed once in PBS$^-$ and then applied to a magnet for 3 minutes. Supernatant was removed and the beads were then resuspended in 250μl of RF-10. Aliquots of 2.0×10^8 patient cells were then added to 250μl (3 beads : 1 CD8 T-cell) of Dyna beads and allowed to incubate for 30 minutes on ice with occasional mixing. After 30 minutes the cell suspension was placed onto a magnet for 3 minutes. The supernatant was then removed placed into a second tube containing 142 μl (1 bead : 1 CD8 T-cell) of Dyna beads. This suspension was placed on ice for an additional 30 minutes with occasional mixing. After 30 minutes cell suspension was placed onto a magnet for 3 minutes. Supernatant was removed and washed once in RF-10.

For co-culture, CD8 depleted PBMC were then added to patient monocytes. Half media changes were done every 7 days for a period of 21 days. Aliquots of 2.5 ml of medium was removed from these cultures and replaced with CD8 depleted donor PBMC in Iscoves containing 10% HuS (Human serum), 5% v/v FCS and 5% w/v IL-2 and 1000U/ml of M-CSF. Harvested supernatants were spun at 1400 rpm for 10 minutes and stored as 1ml aliquots. Cell pellets were lysed in 200μl of lysis buffer for PCR analysis. Infection was quantitated using a p24 EIA Kit.

Cells were harvested from the co-cultures and used to prepare DNA as described above. The *nef* / 3'-LTR region of both virus isolates was amplified by PCR using the above described primer sets and conditions (Example 12). The resulting amplimers were cloned into the plasmid vector pT7T3U19 and the nucleotide sequence determined by the Taq cycle sequencing method with dye-labelled primers.

The C18 macrophage sequence has 3 deletions starting and finishing at positions within 3 nucleotides of the same deletions in $C18_{MBC}$. The encoded *nef* protein is 3 amino acids long compared with 7 amino acids for $C18_{MBC}$. The low homology region of the LTR U 3 region of C18 macrophage is very similar in sequence to $C18_{MBC}$ and similarly it has one extra upstream NFκB site.

On the other hand, the sequence of C98 macrophage has a number of differences from the C98 isolate. While it has exactly the same first deletion of 16 nucleotides just upstream of the polypurine tract (PPT), in the *nef*-alone region, and exactly the same second deletion (position and size) it has an extra deletion of 18 nucleotides at HIV-1_{NL43} equivalent nucleotides 9206 to 9223. The final deletion is in approximately the same position as in the C98 isolate but is 5 nucleotides longer. The encoded *nef* protein is 34 amino acids long compared with 86 amino acids for the C98 isolate. The low homology region is very similar to the C98 isolate, having the same 2 extra upstream NFκB sites and completely lacking the normal 5'-NFκB site.

EXAMPLE 14

Construction and Use of an Infectious Molecular Clone

Molecular biological techniques can be used to construct a molecular clone of, for example, HIV-1 $C18_{MBC}$. Two schemes may be used. In the first scheme genomic DNA, extracted from either the CD4 positive PBMC of the patient C18 or donor PBMC that have been infected with the isolate HIV-1 $C18_{MBC}$, is used as the template for polymerase chain reaction amplification, using thermostable polymerase of high transcriptional fidelity (eg UlTma polymerase or KlenTaq/Pfu polymerase mixture), of long (6 to 7 kb) overlapping fragments representing the 5'- and 3'-parts of the HIV-1 $C18_{MBC}$ proviral genome of total length 9207nts. The amplified fragments may then be ligated together after digestion with a restriction enzyme that cleaves at a unique site common to the overlapping region of the amplified fragments, for example the unique Bgl I or Nco I sites. Ligation of this full length proviral DNA into a plasmid vector will allow its propagation in *E coli* and the subsequent preparation of large (mg) quantities of this molecularly cloned proviral DNA.

In the second scheme donor PBMC that have been infected with the isolate HIV-1 $C18_{MBC}$ are used as a source of non-integrated proviral DNA which can be extracted from the infected cells by the Hirt extraction method (Hirt, 1967). Circular proviral DNA molecules may be linearised by digestion with a restriction enzyme that cleaves at a unique position in the genome (eg the Bgl I or Nco I sites). The resulting linearised molecules can be ligated into a plasmid or, more usually, a bacteriophage lambda (λ) based vector (eg Charon 4a, λWES) after modification of the end to provide blunt or cohesive ends compatible with the vector. Transformation or transduction of *E coli* with the recombinant plasmid or bacteriophage material, respectively allows the propagation of the proviral DNA. Clones of *E coli* containing proviral DNA may be selected and DNA prepared. Molecular clones of retroviral genomes prepared in this way are often permuted. Rearrangement to the functional arrangement of sequences is achieved by restriction enzyme cleavage and religation f fragments to reconstruct the correctly permuted proviral genome.

The molecularly cloned DNA products of both schemes can be used to prepare variant proviral genomes that may be used as the basis of a biologically attenuated HIV-1 vaccine strain. Similarly, they may be modified to contain extra DNA sequences in the *nef*-alone deletion region that may deliver sequences that may be of therapeutic advantage (eg antisense or ribozyme sequences).

Infectious virus particles of HIV-1 $C18_{MBC}$, or modified virus, can be produced by transfection of human cells (eg HeLa cells) which will produce, and release to the culture medium, virus particles of HIV-1 $C18_{MBC}$, or modified virus. These virus particles can be used to infect a variety of CD4 positive cells for further propagation or experimentation.

EXAMPLE 15

In vivo Primate Model

Following construction of infectious molecular clones of the mutant HIV-1 strains, studies are then undertaken in primates to establish attenuation, immunogenicity and vaccine prophylactic efficacy. All studies compare mutant clones of HIV-1 with isogenic wild-type (WT) virus. Initial studies are performed using the macaque (*M. nemistrina*) model of HIV-1 infection. Macaque-infectious WT HIV-1 and mutant clones are compared with respect to duration of viremia, anatomic sites of replication, and cellular and humoral immune responses. Where the mutant HIV-1 clones induce an immune response in the macques infected, challenge studies with WT virus are also performed. Studies are performed in a limited number of chimpanzees, generally in parallel with the macaque studies. Relevant mutations are engineered into WT HIV-1 clones previously shown to produce chronic infection in chimps, and the course of chimp infection with mutant clones compared with historical controls. If infection is established, WT challenge studies is also performed.

Examples 16 to 21 relate to the screening of antibodies in HIV-1 infected individuals to peptides covering regions of Nef.

EXAMPLE 16

Study Subjects

Serum samples were obtained from seven HIV+ve individuals, D-36, C-124, C-98, C-64, C-18, C-49 and C-54. Individuals C124, C-98, C-64, C18, C49 and C-54 (recipients) were infected through units of blood or blood products from donor D36, over a 2-year period. Long-term follow-up of the six recipients and the donor, shows persistent long term asymptomatic infection. This group is referred to herein as the long term non-progressor 1 (LTNP1) cohort. Members of this cohort have been infected for an average of 11 years (10.75 to 14 years) and it was established that the donor had been infected since April, 1981 (Learmont *et al*, 1992). Regular follow-up includes history, physical examination, full blood count, T-cell subset counting and measurement of serum p24 antigen and β2-microglobulin concentrations (Learmont *et al*, 1992; Learmont *et al*, 1995). As controls, sera were obtained from 14 HIV-1 negative individuals (HIV-1-ve), 4 patients who were infected with HIV-1 through sexual activity or through blood transfusion and are also considered long-term non-progressors and 12 HIV-1 positive (HIV-1+ve) individuals that have developed clinical infections (long term progressors, LTP). Sera from patients with autoimmune disease and who were HIV-1-ve were also employed.

EXAMPLE 17

Peptide Synthesis

Peptides corresponding to amino acid residues 1 to 19, 20 to 36, 44 to 65, 72 to 83, 89 to 97, 109 to 114, 121 to 136, 162 to 177, 164 to 186 and 187 to 206 of HIV-1 *nef* (HIV-1_{NL43}) were synthesized using standard t-Boc chemistry and purified by high pressure chromatography as described elsewhere (Fecondo *et al*, 1993).

EXAMPLE 18

Expression of recombinant HIV-1 Nef protein in E. coli

The large scale expression of the 27 kDa form of HIV-1_{NL43} in *E. coli* and subsequent purification were as described by Azad *et al* (1994).

EXAMPLE 19

Screening of sera from HIV-1+ve individuals and control HIV-1-ve groups for reactivity against Nef protein and derived peptides by direct EIA

For detecting antibodies that recognise the Nef protein or its peptide derivatives, highly purified full length Nef protein or peptides corresponding to the HIV-1_{NL43} Nef amin acid sequence were coated onto the wells of 96-well polysyrene microtitre plates at 100 ng/well or 500 ng/well in PBS, respectively. Peptides and proteins were allowed to coat for 2 h at 37°C. After this incubation period the wells were washed three times with PBS containing 0.05% v/v Tween 20 (PBS-Tween) and any remaining available sites on the wells blocked by incubation of 150µl of gelatine (1% w/v) in PBS for 1 h at 37°C. Following washing with PBS-Tween as described above, 50µl of serum diluted in PBS/BSA (1% w/v) was added to the wells and incubated for 1.5 h at 37°C. Sera added to the wells included that from the seven cohort members (D36, C98, C18, C54, C49, C64 and C124 [see Learmont *et al*, 1992]) and the control groups described above. The wells were again washed with PBS/Tween and subsequently incubated with 50µl of biotinylated sheep anti-human Ig (diluted 1:1000 in 1% w/v BSA/PBS; Amersham) for 1 h at 37°C. Following further washing, 50µl of Streptavidin-HRP (diluted 1:1000 in 1% w/v BSA/PBS; Dakopatts) was added to the wells and the plate incubated at 37°C for 30 min. An aliquot of 100µl of substrate (0-phenylenediamine, Sigma) was finally added after washing and the plate allowed to incubate at room temperature for 15 min. The reaction was stopped by the addition of 1 N H_2SO_4 and the plate read at 450/630 nm using a Titertek plate reader.

EXAMPLE 20

Recognition of full length recombinant Nef protein by patient sera

The prevalence of a Nef-specific antibody response in the cohort members (referred to herein as (LTNP1), long term progressors (LTP) HIV-1+ve individuals and another group of long term non-progressors (LTNP2) patients who were infected by different donors was assessed by EIA. Sera obtained from 14 normal individuals (HIV-1-ve) and 14 individuals with autoimmune disease (A/HIV-1-ve) were used as controls.

All individuals who were classified as LTP patients demonstrated high levels f antibodies that recognised full length Nef protein (Figure 12a). Sera obtained from HIV-1-ve or the A/HIV-1-ve groups showed only low level recognition, which was considered at background levels, towards Nef protein (Figure 12b(i)-(iii)). In contrast to normal individuals, sera obtained from the LTNP1 cohort showed high recognition of Nef (Figure 12c), indicating the presence of significant levels of Nef antibodies in the sera of these individuals. The sera titered out to approximately 1:3000. Similar levels of Nef antibodies were observed in the LTP group (Figure 12a). Nef-positive antibodies were also detected in the LTNP2 group and again titered at 1:3000 dilution (Figure 12d).

EXAMPLE 21

Recognition of Nef-derived peptides by sera

Recognition of synthetic peptides, which correspond to amino acid sequences of Nef, by the LTNP1 cohort was assessed. Various peptides were assessed to detect those antigenic epitopes of Nef protein recognised by these individuals. Peptides corresponding to amino acid sequences 1 to 19; 20 to 36; 44 to 65; 72 to 83; 89 to 97; 109 to 114; 164 to 186; 187 to 206; 121 to 135 and 162 to 177 of HIV-1_{NL43} Nef protein were used to screen sera for the presence of specific antibodies. All sera from the LTP group recognised all Nef-derived peptides tested (Figure 13a(i)-(x)). Sera titered between 1:1000 and 1:10,000. Sera from patients with autoimmune disease displayed only low background non-specific recognition. Normal sera from HIV-1-ve individuals tested to date also displayed only background activity (Figure 13b). Sera from the LTNP1 and LTNP2 groups also showed significant reactivity against Nef peptides corresponding to Nef amino acid sequences 1-19, 20-36, 44-65, 72-83, 89-97, 109-114, 121-135, 164-86 and 187-206 (Figure 13c(i)-(x) and d(i)-(x)). Sera from the LTNP2 group also showed significant reactivity against Nef peptide 162-177 (Figure 13d(i)-(x)), similar to that showed by the LTP group, indicating that this region of Nef was immunogenic. However, sera from the LTNP1 cohort showed no significant reactivity towards peptide 162-177 above background levels obtained with normal HIV-1-ve sera (Figure 13c(i)-(x)), indicating that this group f individuals were exposed to cells expressing a Nef protein which did not contain this region. While sera from the

LTNP1 cohort did not react with the peptide corresponding to amino acid residues 162-177 of Nef, the sera from all patients did recognise a longer peptide, 164 to 186, which encompassed most of Nef 162-177. This clearly indicates that the sera recognised antigenic epitopes between 177-186.

These results clearly indicate that all individuals from the LNTP1 cohort were exposed at some time to HIV-1 infected cells expressing a Nef protein that only had a small deletion encompassing amino acids 162 to 177.

The antibody testing has identified an antigenic region in the Nef protein which if deleted gives rise to attenuated HIV-1 viral strains. Hence, testing of the HIV-1 positive population may identify further examples of individuals infected with attenuated viral quasispecies. Additionally, lack of recognition of this antigenic epitope offers an antibody assay for testing animals experimentally infected with an HIV-1 *nef* attenuated viral strain, in particular deleted in the region covering Nef amino acids 162 to 177 (relative to HIV-1_{NL43} Nef).

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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